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Division VI—Volume 2

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION

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BIOLOGICAL EFFECTS
OF EXTERNAL RADIATION

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FOREWORD

Since the discovery of practical means of utilizing the energy of the atomic nucleus, a large and complex atomic energy industry has begun in the United States. As a result of conditions in the world, external to the United States, the requirements of national security have been paramount in our development of this industry thus far. Constant and increasing attention, however, has been given to the problems of economic nuclear power and to the medical and industrial applications of radioactive materials with a view toward "improving the public welfare, increasing the standard of living, strengthening free competition in private enterprise, and promoting world peace." To this end the Atomic Energy Commission has sought the most effective means to accelerate the practical exploitation of nuclear data by American science and industry. The National Nuclear Energy Series is designed to provide for scientists and engineers as comprehensive a source of such data as is possible. The scope of the information presented in these volumes is a measure of American achievements to date in the field of atomic science.

Lewis L. Strauss, Chairman
U. S. Atomic Energy Commission

ACKNOWLEDGMENT

The Manhattan Project Technical Section of the National Nuclear Energy Series embodies results of work done in the nation's wartime atomic energy program by numerous contractors, including Columbia University. The arrangements for publication of the series volumes were effected by Columbia University, under a contract with the United States Atomic Energy Commission. The Commission, for itself and for the other contractors who contributed to this series, wishes to record here its appreciation of this service of Columbia University in support of the national nuclear energy program.

PREFACE

This volume is one of a series which has been prepared as a record of the research work done under the Manhattan Project and the Atomic Energy Commission. The name Manhattan Project was assigned by the Corps of Engineers, War Department, to the far-flung scientific and engineering activities which had as their objective the utilization of atomic energy for military purposes. In the attainment of this objective, there were many developments in scientific and technical fields which are of general interest. The National Nuclear Energy Series (Manhattan Project Technical Section) is a record of these scientific and technical contributions, as well as of the developments in these fields which are being sponsored by the Atomic Energy Commission.

The declassified portion of the National Nuclear Energy Series, when completed, is expected to consist of some 60 volumes. These will be grouped into eight divisions, as follows:

Division I — Electromagnetic Separation Project
Division II — Gaseous Diffusion Project
Division III — Special Separations Project
Division IV — Plutonium Project
Division V — Los Alamos Project
Division VI — University of Rochester Project
Division VII — Materials Procurement Project
Division VIII — Manhattan Project

Soon after the close of the war the Manhattan Project was able to give its attention to the preparation of a complete record of the research work accomplished under Project contracts. Writing programs were authorized at all laboratories, with the object of obtaining complete coverage of Project results. Each major installation was requested to designate one or more representatives to make up a committee, which was first called the Manhattan Project Editorial Advisory Board, and later, after the sponsorship of the Series was assumed by the Atomic Energy Commission, the Project Editorial Advisory Board. This group made plans to coordinate the writing programs at all the installations, and acted as an advisory group in all matters affecting the Project-wide writing program. Its last meeting was held on Feb. 9, 1948, when it recommended the publisher for the Series.

The names of the Board members and of the installations which they represented are as follows:

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University of Rochester	D. R. Charles, M. J. Wantman

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* Represented Madison Square Area of the Manhattan District.

† The Y-12 plant at Oak Ridge was operated by Tennessee Eastman Corporation until May 4, 1947, at which time operations were taken over by Carbide & Carbon Chemicals Corporation.

‡ Clinton Laboratories was the former name of the Oak Ridge National Laboratory.

§ SAM (Substitute Alloy Materials) was the code name for the laboratories operated by Columbia University in New York under the direction of Dr. H. C. Urey, where much of the experimental work on isotope separation was done. On Feb. 1, 1945, the administration of these laboratories became the responsibility of Carbide & Carbon Chemicals Corporation. Research in progress there was transferred to the K-25 plant at Oak Ridge in June, 1946, and the New York laboratories were then closed.

Many difficulties were encountered in preparing a unified account of Atomic Energy Project work. For example, the Project Editorial Advisory Board was the first committee ever organized with representatives from every major installation of the Atomic Energy Project. Compartmentation for security was so rigorous during the war that it had been considered necessary to allow a certain amount of duplication of effort rather than to permit unrestricted circulation of research information between certain installations. As a result, the writing programs of different installations inevitably overlap markedly in many scientific fields. The Editorial Advisory Board has exerted itself to reduce duplication in so far as possible and to eliminate discrepancies in factual data included in the volumes of the NNES. In particular, unified Project-wide volumes have been prepared on Uranium Chemistry and on the Analysis of Project Materials. Nevertheless, the reader will find many instances of differences in results or conclusions on similar subject matter prepared by different authors. This has not seemed wholly undesirable for several reasons. First of all, such divergencies are not unnatural and stimulate investigation. Second, promptness of publication has seemed more important than the removal of all discrepancies. Finally, many Project scientists completed their contributions some time ago and have become engrossed in other activities so that their time has not been available for a detailed review of their work in relation to similar work done at other installations.

The completion of the various individual volumes of the Series has also been beset with difficulties. Many of the key authors and editors have had important responsibilities in planning the future of atomic energy research. Under these circumstances, the completion of this technical series has been delayed longer than its editors wished. The volumes are being released in their present form in the interest of presenting the material as promptly as possible to those who can make use of it.

The Editorial Advisory Board

The Manhattan Project Technical Section of the National Nuclear Energy Series is intended to be a comprehensive account of the scientific and technical achievements of the United States program for the development of atomic energy. It is not intended to be a detailed documentary record of the making of any inventions that happen to be mentioned in it. Therefore, the dates used in the Series should be regarded as a general temporal frame of reference, rather than as establishing dates of conception of inventions, of their reduction to practice, or of occasions of first use. While a reasonable effort has been made to assign credit fairly in the NNES volumes, this may, in many cases, be given to a group identified by the name of its leader rather than to an individual who was an actual inventor.

UNIVERSITY OF ROCHESTER PROJECT FOREWORD

The University of Rochester Manhattan Project had its inception on April 5, 1943, with the appointment of Dr. Stafford L. Warren,* Professor of Radiology and Chairman of the Department of Radiology at the University of Rochester School of Medicine and Dentistry, as Consultant to the Manhattan Engineer District (later as Chief of the Medical Section). Under his guidance and direction the local project was established and its operational policies formulated. On November 2, 1943, Dr. Warren was commissioned colonel in the Army Medical Corps, and the subsequent responsibility for the Project was assumed by the present Director on November 13, 1943.

In many respects the atmosphere of the work was in marked contrast to the academic freedom of a university environment. The research was frequently of applied rather than of fundamental nature, though the latter was by no means lacking. In addition to physical and spiritual isolation from our accustomed confreres, we found ourselves surrounded by a multitude of security, Army, governmental, and war-manpower regulations, but the majority of the personnel made the necessary mental adjustments without undue hardship and with commendable reasonableness and good grace. Not infrequently we found these apparent handicaps working to our mutual advantage.

The organization of the Project was likewise unusual and, to a certain extent, experimental. To accomplish the task in the specified time and to utilize effectively experienced personnel made scarce by previous demands of the war, individuals were placed in positions where their capabilities could produce maximum benefit to the Project as a whole. To this end, ten autonomous but mutually interdependent divisions were established, whose coordination was effected through the Director's Office so that priorities on material, manpower, and concentration of effort could be channeled in the proper direction with the shifting phases of the various problems. Experiments were discussed and organized on a cooperative basis through a system of "planning sessions" in such a manner that the expert opinion of participating members of specialized divisions could make major contributions to the structure of many of the experiments. This procedure also enabled the divisions of Pathology, Hematology, and Statistics to coordinate their activities with other divisions so that they could analyze properly the mass of experimental data which must, of necessity, pass through their respective laboratories.

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*Now Dean of the Medical School, University of California at Los Angeles.

It would be misleading to aver that this system of research procedure presented here was without fault and not beset by difficulties. It is not an easy matter for an investigator to reconcile his ideas and personal ambitions with those of a group for a common objective, especially when frequently his entire training and previous progress have been based upon individual achievement. Particularly is this true when he has not had the privilege of participating in the selection of his associates. The system, however, worked surprisingly well under somewhat unfavorable circumstances and is worthy of further exploration under peacetime conditions.

It is impossible to pay proper tribute to the many individuals—scientific, technical, and nontechnical—who participated in this endeavor. Neither can one, by reading the following pages, appreciate fully the mental and physical labor that went into the enterprise. Approximately two million man-hours were required to produce the research from which these volumes are derived.

The Administrative Office wishes to express its appreciation to the Project personnel for their confidence and loyalty; to the University as a whole for its support and cooperation; to the many Rochester industries and businesses for the materials supplied and the services rendered; to the Area Engineer's Office for its aid in facilitating the conduct of the program; to Dr. Ellice McDonald, Director of The Biochemical Research Foundation of the Franklin Institute, at Newark, Del., for his cooperation in coordinating the research under his contract with that of Rochester.

Andrew H. Dowdy, M.D.
Professor of Radiology and Director of the University of Rochester Project, 1943-1947; now Professor of Radiology, School of Medicine, University of California at Los Angeles

VOLUME EDITOR'S PREFACE

The studies reported in this volume represent the major part of the work done at the University of Rochester during the war period on the biological effects of X radiation along with a collaborative study of the chronic effects of neutron irradiation with the Biochemical Foundation, Newark, Del.

The experiments were planned with the objectives of obtaining a description of the lethality and of the pathological and genetic effects of both acute and chronic irradiation and of developing clinical criteria for detection of minimal effects of radiation in exposed personnel. Progress was made toward the former objectives, but no useful clinical criterion for detection of effects of low levels of whole-body irradiation were obtained at that time, the changes in finger ridge detail being associated with rather large dosage to the hands.

It was proposed originally, before the results of parallel investigations elsewhere had become available through declassification, to add a summarizing and integrating chapter stating the general implications of this work as they were seen at that time. Delays in preparation and publication permitting the appearance of other reports allowed it to become evident that only more general integration would be of real value and that this would require a separate work. Consequently, each chapter refers separately to other investigations according to the results available when it was written.

As subjects for research, the biological effects of ionizing radiation, particularly in the larger organisms, appear to be very complex, as no doubt they are. Much of the apparent complexity, however, probably arises from the fact that this is a period of accumulation of data with few generalizations either to correlate experiments or guide their direction. There seems to be no doubt, however, as is already evidenced in genetics, that studies of the effects of radiation will ultimately extend knowledge of biological processes far beyond the limited objectives of therapeutic radiology or the establishment of safe levels of human exposure.

Henry A. Blair

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PART I

SINGLE DOSES OF WHOLE-BODY X RADIATION

Chapter 1*

STUDIES ON THE EFFECTS OF MASSIVE DOSES OF X RADIATION ON MORTALITY IN LABORATORY ANIMALS

By Robert D. Boche and Francis W. Bishop

The object of these experiments was to supplement the existing literature on the effects of single acute exposures of animals and to form an estimate of the variations that exist among different mammalian species. The applicability to man is uncertain, but it may be assumed, as a first approximation, that his sensitivity lies within the range of other species. Tissue studies on animals sacrificed serially have been carried out for the rat, and fairly complete histological studies have been made on animals at death. This material is presented in Chap. 2. Systematic studies have been made on the behavior of peripheral-blood elements in the rat, dog, and monkey following single exposures, and these data are the subject of Chap. 3.

No attempt is made here to review the large volume of literature on the effects of radiation on mortality in various organisms since this subject is given comprehensive treatment in the National Nuclear Energy Series, Division IV, Volume 22I. It is now well established that curves relating mortality to dose are of the sigmoid type for most organisms studied,[1] and mammals are no exception in this respect. These sigmoid curves may be conveniently transformed to straight lines by the method of Bliss,[2] which is based on the assumption of normally distributed susceptibility of the individuals radiated and usually geometric increase in effect with increasing dose. Such straight lines provide the simplest method of estimating the median lethal dose (LD_{50}) for a given set of conditions, and variations in LD_{50} values constitute a convenient basis for comparison of species or other factors. This is illustrated for survival of dogs given 1000-kv radiation at various intensities and doses. A group of 84 mongrel dogs were randomized so as to distribute the variations in age, sex, length of time in the kennels, and other uncontrolled factors equally into each of five groups. These dogs were given various doses of radiation, with the results shown in Table 1.1.

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*Based on Report MDDC-250 (II-188-5980).

Table 1.1 — Thirty-day Mortality and Average Survival Times in Dogs Exposed to Single Doses of 1000-kv X Radiation*

Dose, r	Number of dogs	Deaths	Mortality, %	Average survival time, days
200	16	1	6.25	18
300	19	6	31.6	17.7
400	20	16	80.0	15.8
500	20	20	100.0	13.5
600	9	9	100.0	12.9

*Irradiation time, 44 min for all dosages; half-value layer (HVL), 5.12 mm Pb.

The data of Table 1.1 are represented graphically in Figs. 1.1 and 1.2, which illustrate the increase in mortality with dose for a 30-day interval. Substantially all the information is obtained in 30 days since the dogs were followed for 6 weeks and only one death occurred after 30 days. Figure 1.2 illustrates the point that with increasing dose not only do more dogs die but they also die sooner. This is also true of the rat, as is shown in Fig. 1.3 for a series of 83 rats which died after doses varying from 400 to 1500 r (LD_{50}, 550 r) of 250-kv radiation [half-value layer (HVL), 2.1 mm Cu] given at approximately 6 r/min.

A small-scale experiment on the influence of age and sex on susceptibility was carried out. Two hundred forty rats of the Wistar strain were prepared, randomized into four groups, and given 375, 500, 625, and 750 r of 250-kv radiation (HVL, 2.1 mm Cu). The dosages were all administered in 44 min. The young animals were 2 months ±5 days of age, and the older ones were 7.5 months ±5 days. The structure of the experiment and the results are presented in Table 1.2.

The experiment will be recognized as factorial in design. The data may be classified according to age, sex, dose, and interaction. The percentage mortality among adults was 34.2; among young rats, 31.6; among males, 39.1; and among females, 31.6. The differences among categories are not significant, i.e., females may be more resistant

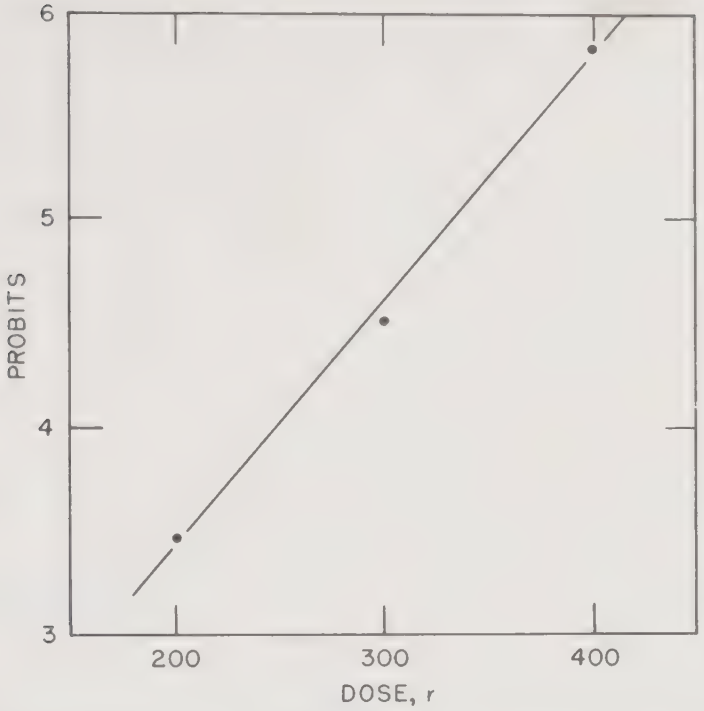

Fig. 1.1—The relation between mortality and dose for dogs given single exposures of 1000-kv radiation.

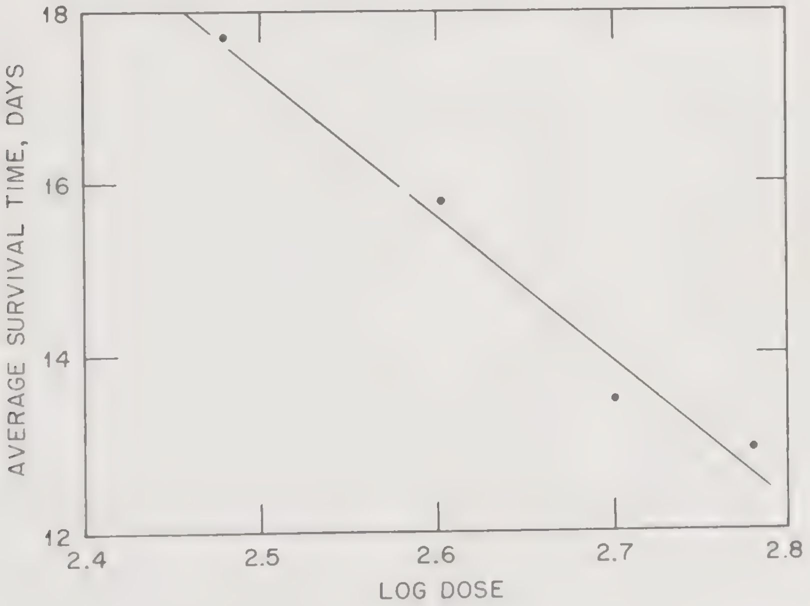

Fig. 1.2—Average survival times for groups of dogs exposed to various doses of 1000-kv radiation.

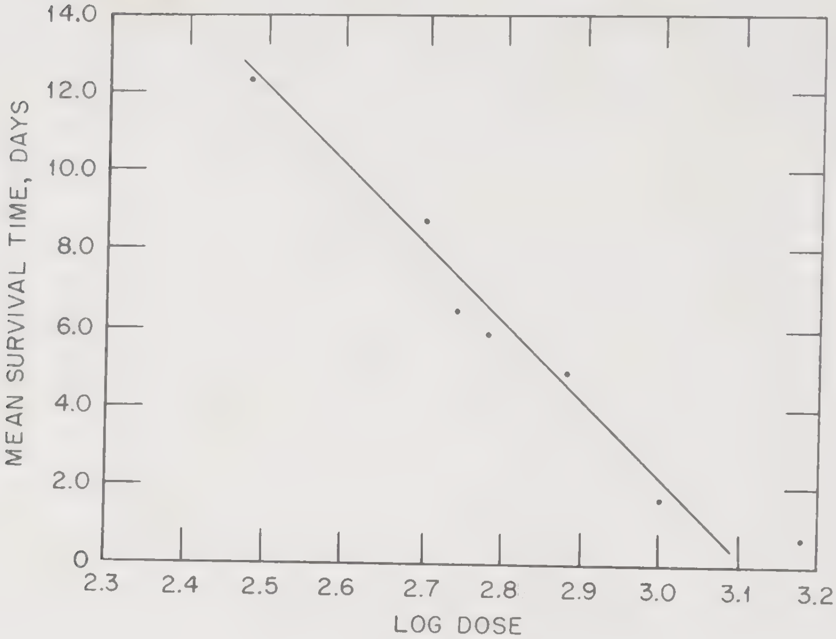

Fig. 1.3—Relation between mean survival time of dying animals and varying dosage for rats.

Table 1.2—Influence of Age and Sex on Susceptibility in the Rat

Dose, r	Sex	Age	Number of animals	Number of deaths in 30 days
375	Males	Young	15	0
		Old	15	0
	Females	Young	15	0
		Old	15	1
500	Males	Young	15	1
		Old	15	2
	Females	Young	15	2
		Old	15	1
625	Males	Young	15	9
		Old	15	7
	Females	Young	15	1
		Old	15	5
750	Males	Young	15	13
		Old	15	15
	Females	Young	15	12
		Old	15	10

than males, but this experiment does not indicate a very great difference. The relation between mortality and dose (250 kv) for these animals combined is shown in Fig. 1.4.

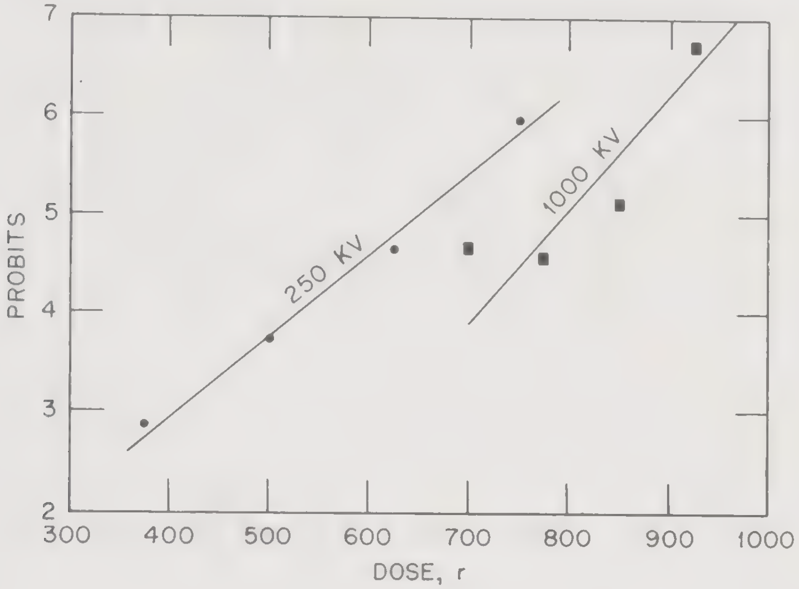

Fig. 1.4—Relation between mortality in the rat and dosage for 250- and 1000-kv X radiation.

Considerations of specific ionization lead to the expectation that high-energy X radiation would be less effective per roentgen than lower-energy radiation in producing biological effects. This has been shown clearly by Packard[3] for Drosophila eggs and is confirmed here for the rat. Packard found that 900-kv radiation produced only about 80 per cent of the effect of 200-kv radiation when both were measured with Victoreen ionization chambers. Figure 1.4 also shows the results of exposures of rats to 1000-kv compared to exposures to 250-kv radiation. Intensities were adjusted in each case so that the times in which the doses were given were identical, i.e., 44 min. Although there is some uncertainty about the slope and position of the 1000-kv line, it is clear from these and other data that the LD_{50} is in the neighborhood of 800 r for 1000-kv radiation. The ratio of effectiveness of 250- and 1000-kv radiations is therefore approximately 650 to 800, or 0.81, in good agreement with Packard's results on Drosophila eggs.

For purposes of comparison with the rat and dog a series of hamsters was irradiated with 250-kv X rays, again in 44 min for all doses. The 30-day mortalities are shown in Table 1.3.

Table 1.3 — Mortality in Hamsters Following X Radiation

	Mortality		
Dose, r	Males	Females	Total
550	2/14	1/15	3/29
625	5/14	1/15	6/29
700	6/12	5/15	11/27
775	12/13	10/14	22/27
850	7/7	6/7	13/14

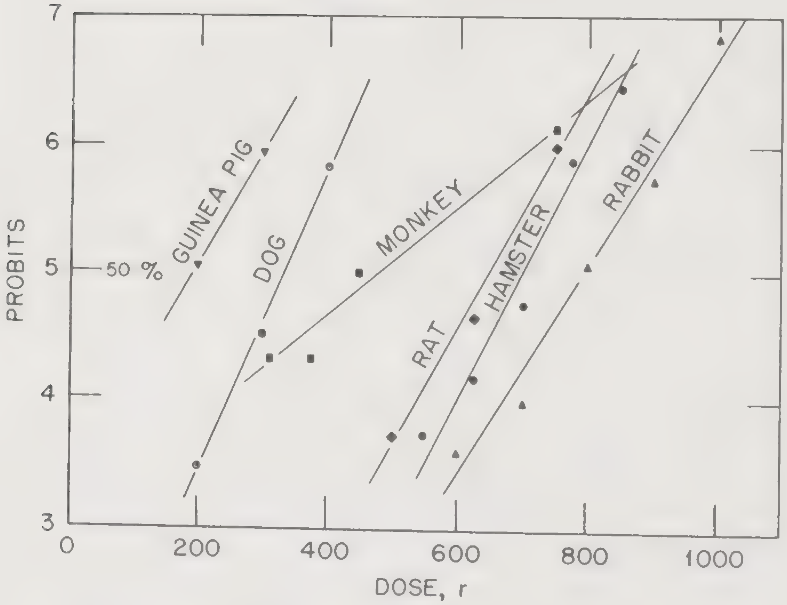

Fig. 1.5 — Comparative data on the relations between mortality and X-ray dosage for laboratory mammals (compiled from various sources).

The data of Table 1.3 are shown graphically in Fig. 1.5, which indicates that the LD_{50} for the hamster is in the vicinity of 700 r for 250-kv radiation.

A small-scale exploratory experiment was carried out in this laboratory by H. C. Stebbins on monkeys (Macacus rhesus). The animals were given 250-kv X radiation at 3.5 r/min and were observed for 6 to 8 weeks before sacrifice. The results of the experiment are given in Table 1.4.

Figure 1.5 also shows the dosage-mortality curves from various sources for a series of representative mammals including those discussed above. All curves are for 200- to 250-kv radiation except that for the dog. Data for the guinea pig are from Ellinger;[4] those for the rabbit are from Hagen et al.[5] LD_{50} values from various sources are shown in Table 1.5. The agreement of data from various sources is good considering that strain differences within a species are known to affect susceptibility.

Table 1.4 — Mortality in Monkeys Following X Radiation

Dose, r	Mortality		Average survival time, days
	Males	Females	
300	0/2	0/2	
325	1/1	1/3	13
375	0/4	2/4	38
450	2/4	2/4	16
750	5/6	2/2	17
850	5/5	3/3	11
1500	2/2	2/2	6.5
2000	3/3	1/1	6.5

Table 1.5 — Estimates of the Lethal Dose for Various Species

Species	LD_{50}, r	Radiation, kv	Reference
Guinea pig	200	200	4,6
Dog	300	200	7
Dog	335	1000	*
Mouse	400	200	4
C_3H mouse	450	200	6
LAF mouse	600	200	6
Monkey	500	250	*
Rat	590	200	7
Rat (Wistar)	650	250	*
Hamster	700	250	*
Rabbit	790	200	5

*Data from experiments reported in this chapter.

Interest in very high-intensity sources prompted a few preliminary experiments of this sort with rats. Doses of 850 r of 1000-kv radiation were administered in 1.5 min to 70 rats; the 30-day mortality was 38.5 per cent. However, 850 r given to 25 rats over the course of 2 hr led to a mortality of 24 per cent. It was further found that the LD_{50} at intensities of 550 r/min was in the vicinity of 800 to 850 r, in agreement with data of Fig. 1.4 for intensities of approximately 18 r/min. Evidently, increasing the intensity eightyfold does not change the order of magnitude of effect. It seems improbable that a further increase in intensity by ten- or a hundredfold will produce strikingly different effects, although this is worth investigation.

Reference has been made to the fact that age and sex do not markedly affect susceptibility in the rat. To this may be added the fact that withholding food or water, or both, for 40 hr before exposure does not influence survival in the rat, nor does the administration* of liver extract or vitamin B_6.HCl (0.1 mg/140 g) before or after exposure.

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*These studies were made by K. Fink.

Chapter 2*

PATHOLOGICAL CHANGES EXHIBITED BY ANIMALS EXPOSED TO SINGLE DOSES OF X RADIATION

By Roger G. Metcalf, Richard J. Blandau, and Thomas B. Barnett

ABSTRACT

Albino rats were exposed to a single dose of 550 r of whole-body radiation and were sacrificed at intervals of from 15 min to 42 days after exposure. Sections of selected organs were examined microscopically, and studies were made on the peripheral blood and bone marrow and on sperm from the vas deferens.

Within the first hour after irradiation, cellular degeneration was observed in the lymph nodes, spleen, and bone marrow. These organs subsequently showed a marked reduction in cellularity. Regeneration resulted in a return to normal in the lymph nodes by the seventeenth day and in the spleen and bone marrow by the fortieth day.

In the testes, degeneration of spermatogonia occurred within the first few hours, and shortly thereafter there occurred a depletion of spermatogonia and later a disappearance of the remainder of the spermatogenic elements as the process of maturation continued. Spermatogonia began to reappear between the twentieth and fortieth days.

Spermatozoa from the vasa deferentia exhibited no change in motility prior to the twenty-first day, but after this time motility was reduced or absent in most cases.

Degenerative changes were observed in the granulosa cells of the ovarian follicles as early as 1 hr after exposure.

Degeneration of epithelial cells in the mucosa of the gastrointestinal tract occurred very early (1 to 2 hr). These changes were par-

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*Based on Report UR-123, July 17, 1950; work performed by Roger G. Metcalf, Thomas B. Barnett, George M. Suter, and Morey J. Wantman.

ticularly marked in the small intestine, but in no case was ulceration noted. For a period of about 60 hr there was a reduction in mitotic activity in the mucosal epithelium and an alteration in the epithelial cells themselves, but by the end of 60 hr the appearance of the intestinal mucosa had returned to normal.

In the peripheral blood the lymphocytes were first affected. They were significantly reduced in number as early as 15 min after exposure and by 15 hr had almost entirely disappeared. Recovery occurred between the tenth and forty-first days. After an initial slight increase in circulating neutrophils, there developed a neutropenia that persisted for several days and then gradually returned to normal.

Anemia was first observed at approximately 100 hr and was most severe on about the fifteenth day. The red blood cells and hemoglobin levels returned to normal between the thirty-second and fortieth days.

In the bone marrow the erythrogenic series showed a reduction in numbers earlier than did the myeloid group. Regeneration was also more rapid in the red-cell precursor group.

Megakaryocytes were markedly reduced between the fourth and the twelfth days. This reduction was paralleled by a drop in the number of circulating platelets. It was during this period that microscopic hemorrhage most often occurred in lymph nodes and bone marrow and that anemia was most profound.

Although 25 per cent of the rats died after being exposed to 550 r, this does not represent an accurate mortality figure for this dosage level since many of the animals were sacrificed prior to the period of highest death rate. Most of the deaths were attributed to septicemia.

1. INTRODUCTION

Heineke,[1] working in the Surgical Clinic in Leipzig in 1904, made the first careful histological studies on the blood and blood-forming organs in mice, guinea pigs, and rabbits following exposure to X rays. Since then there have been numerous reports on the effects of irradiation on selected groups of body tissues or organs but few reports on the effects of a standardized dose of X rays on various tissues after high-voltage whole-body irradiation. Probably the most extensive studies of whole-body irradiation are those of Kolodny[2] on rabbits and of Henshaw, Thompson, and Meyer[3] on mice, in which the effects on tissues such as liver, kidney, spleen, and intestine are described.

Nettleship[4] and Langendorf[5] have exposed rodents to whole-body irradiation to determine the effects of X rays on selected tissues and organs.

No investigations are reported in which a series of animals had been exposed to high-voltage whole-body irradiation and then sacrificed at frequent intervals over long periods of time in order to determine the sequence of injury and repair to body tissues as a whole and at the same time to correlate the changes in the peripheral blood with those in the bone marrow.

In view of the obvious need for a broader and more detailed understanding of the manner in which high-voltage irradiation affects a living organism as a whole, it was decided to investigate the effects of whole-body irradiation in a series of rats that could be sacrificed at short intervals and whose tissues, including the peripheral blood, could be studied in greater detail than had previously been undertaken.

2. MATERIALS AND METHODS

In this study, 374 young albino rats were exposed in small groups to a dose of 550 r of whole-body X irradiation using 250 kv and 15 ma with a target skin distance (TSD) of 42 in. Aluminum parabolic filters with 0.5 mm of copper were used. The half-value layer (HVL) was 2.1 mm Cu. The length of exposure was approximately 1 hr 15 min.

These animals were killed by means of an overdose of ether at intervals of 15 min during the first hour, at intervals of 30 min from the first to the eighth hours, at intervals of 1 hr from the ninth to the forty-fourth hours, at intervals of 6 hr from the fiftieth to the 122d hours, and at daily intervals from the sixth to the twentieth days. The remaining animals were killed on the twenty-eighth, thirty-fifth, and forty-second postexposure days. At least two control rats were sacrificed at each interval, and the same procedures were applied to them as to the experimental rats. Peripheral-blood studies on the experimental animals were carried out just before exposure to X rays and again just prior to sacrifice. A group of 88 animals was used exclusively for peripheral-blood studies after having been exposed to 550 r of whole-body irradiation. Their peripheral blood was then examined at approximately ½-hr intervals for the first six postexposure days. No animal was bled more than seven times, including the preexposure bleeding. An additional group of 16 exposed rats was bled at daily intervals from the sixth to the twenty-first days and on the twenty-eighth, thirty-fifth, and forty-second days.

Peripheral-blood studies included (1) hemoglobin as determined by the Klett-Summerson method, (2) white blood cell count and differential count, (3) red blood cell count, (4) blood platelet count, and (5) reticulocyte count. The general procedures in the peripheral-blood studies were carried out according to standard methods and need not be described in detail.

From 193 of the experimental animals, tissue samples were taken of lung, liver, kidney, spleen, regional lymph nodes, ovary or testes, bone marrow from a vertebra and distal femur, and each portion of the digestive tract except the esophagus. The tissues were immediately fixed in Zenker's formol solution and subsequently embedded in paraffin, cut at 10 μ, and stained in hematoxylin and eosin.

The experiment was so designed that approximately one-half the animals were males and one-half females. Sperm examinations were made in all the males immediately following sacrifice and consisted in squeezing the contents of the vasa deferentia and cauda epididymides into a drop of Locke's solution kept at 37°C. This suspension was examined microscopically for sperm motility.

Bone-marrow smears were made in 270 irradiated and control animals by the following method: Marrow was removed from the shaft of the femur and suspended in dog serum. Smears of this suspension were drawn by the cover-slip method and stained with Wright's stain. Subsequently, differential marrow-cell counts were recorded; at least 200 cells were included in each count.

The animals that died were autopsied as soon after death as possible, and tissues were removed and prepared for subsequent histological examination.

3. OBSERVATIONS

The histopathological findings in the various tissues removed from the experimental animals are described in this section.

3.1 Spleen. Thirty minutes after irradiation, nuclear pycnosis fragmentation in the peripheral areas of the splenic follicles was considerably more prominent than in the controls. During the first 2 hr the lymphocytes immediately surrounding the arterioles showed much less evidence of injury than those farther removed from the vessels, but, as the postirradiation interval lengthened, there was a gradual decrease in the number of normal lymphocytes surrounding the arterioles. Areas of injury were evidenced by the clumping of the chromatin material within the nuclear membrane and the eventual loss of that membrane, resulting in the fragmentation and dissemination of the nuclear material. The cytoplasm of the injured cells became quite granular and lost its normal staining quality. Also at this time the sinusoids of the red pulp were dilated, and their endothelial cells were swollen. At 45 min after irradiation, many cells in the splenic cords began to show definite injury.

A rapid decrease in the size of the splenic follicles was evident by the eighth postexposure hour and became progressively more so up to the thirtieth hour. Macrophages were present in the splenic follicles in great numbers. They were filled with clumped nuclear fragments and blood pigments and appeared to be transporting these materials to the more peripheral sinusoids. The polymorphonuclear leucocytes had also increased above normal numbers. By the twentieth postexposure hour great progress had been made in the removal of the cellular debris, and the macrophages were much less evident than earlier. The progressive reduction in size of the follicles appeared to be associated with the removal of cellular debris (Fig. 2.1). Scattered aggregates of hematopoietic cells are normally seen in the spleens of rats. In the experimental animals, as the number of cells in the red pulp decreased, the small aggregates of erythroblasts and normoblasts became more obvious.

The spleens of animals removed 25 hr after irradiation showed a noticeable reduction in the number of cells in the red pulp and in the number and size of the splenic follicles (Fig. 2.2). By 30 hr after irradiation the spleen was almost devoid of follicles, the cells of the red pulp had decreased to a minimum, and the connective tissue of the spleen was more apparent by contrast (Fig. 2.3).

Between the thirtieth and thirty-fifth hours after irradiation there was an indication of the return of lymphocytes to the splenic follicles. The site of the first return of these cells in the irradiated nodes was varied. In some regions the cells first appeared immediately adjacent to the central artery, but in others they made their appearance in the regions corresponding to those in which the earliest cellular fragmentation occurred. In general, the return of the lymphocytes to the spleen was slow up to the 200th postexposure hour. After this time their number increased at a moderate rate, but the return of the cells to the red pulp was not marked until after the fifteenth postexposure day.

During the period between the sixteenth postexposure day and the termination of the experiment (forty-second day) the spleens of the experimental animals showed a definite hypercellularity, which has been interpreted as evidence of an increase in extramedullary myelopoiesis.

Accumulations of considerable amounts of brown pigment were observed more frequently in the spleens of the experimental animals killed between the third and thirty-fifth days after irradiation.

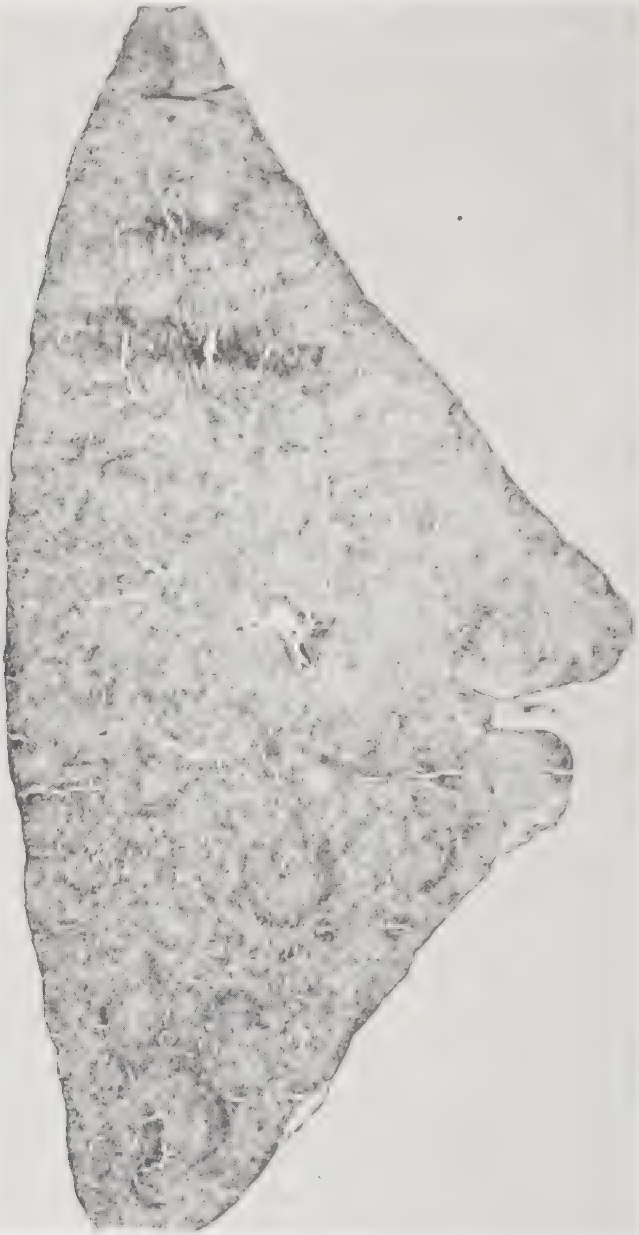

Fig. 2.1—Spleen of the rat removed approximately 20 hr after irradiation. The number and size of splenic follicles are reduced. 20×

Megakaryocytes were present in greater numbers in the spleens of animals examined after the eighteenth postexposure day than in the spleens of the controls.

3.2 Lymph Nodes. The sequence of events during the degenerative and regenerative phases of lymph nodes injured by whole-body irradiation followed closely that described for the spleen.

One hour after irradiation a great number of cells in the germinal centers were noticeably swollen, and many of them showed karyorrhexis and karyolysis. Figure 2.4 is a low-power photomicrograph of a portion of a submaxillary lymph node from an animal killed 2 hr after irradiation. The extensive pycnosis and the disruption of parenchymatous elements throughout the node are clearly evident. Figure 2.5 is a higher magnification of a follicle from the lymph node shown in Fig. 2.4 and more clearly shows the large mononuclear cells invading the follicle and engulfing the cellular debris. The rapid progression of injury is further shown in Fig. 2.6, which depicts a lymph

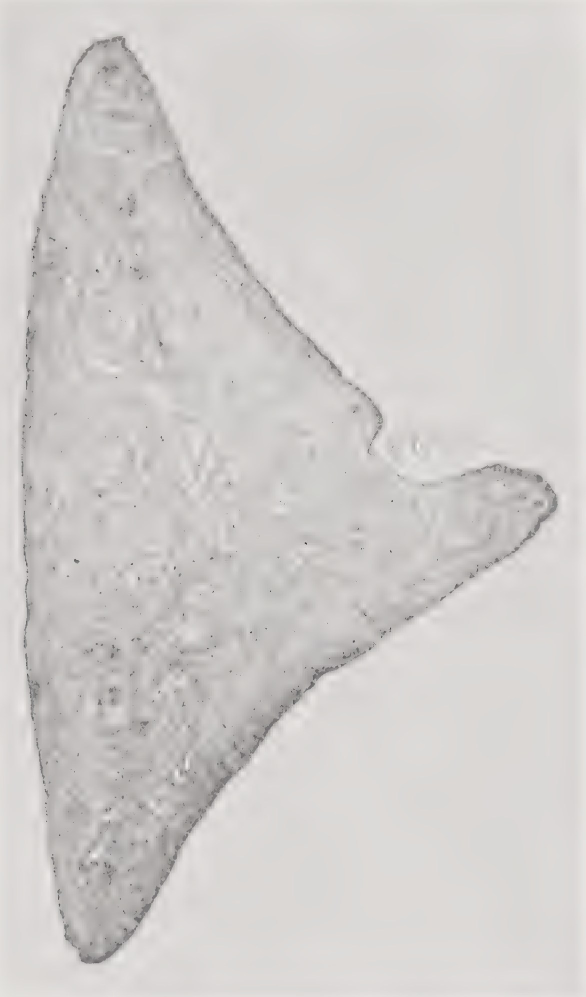

Fig. 2.2—Spleen of the rat removed 25 hr after irradiation. The splenic follicles are further reduced in size, and their number is rapidly decreasing. 20×

follicle removed 4 hr after irradiation. The follicle is reduced in size, and the perifollicular cells show marked pycnosis. The number of macrophages has increased greatly. By 6 hr after irradiation, cy-

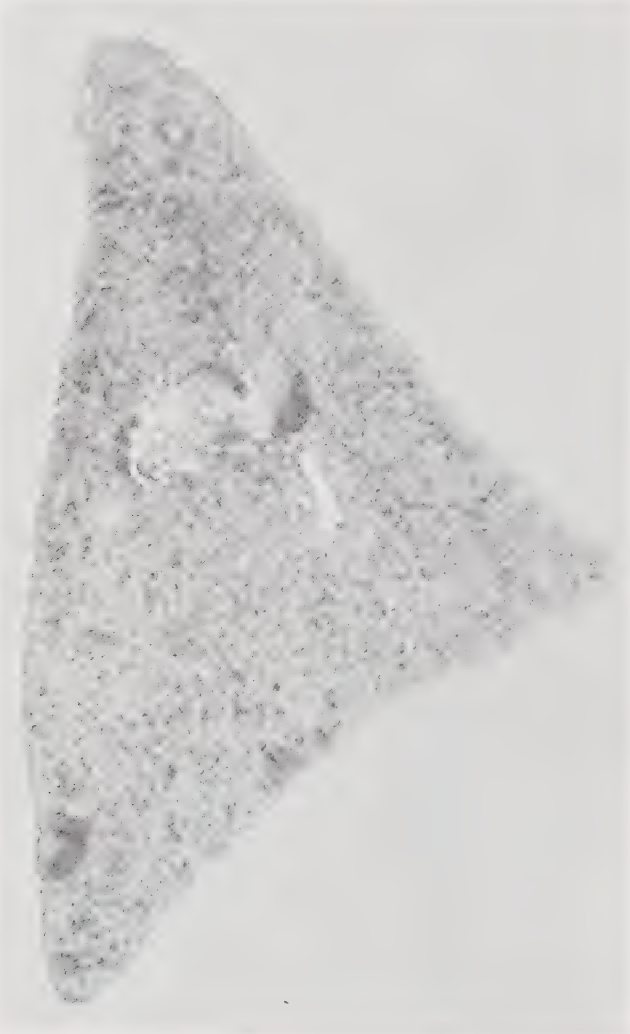

Fig. 2.3—Spleen of the rat removed approximately 30 hr after irradiation. The splenic follicles are completely absent, and the amount of connective tissue has increased. Regeneration of the spleen is just beginning. 20×

tolysis had reached its maximum, and the follicles were filled with necrotic material that was rapidly being removed toward the medulla by the large mononuclear cells. At this time there was also a definite indication of injury to the cells in the remainder of the cortex. Between 6 and 15 hr there was a marked reduction in the cortical thickness and a corresponding increase in the volume of the medullary area. The reduction in the extent of the cortex was due to the disappearance of the lymphatic follicles, whereas the medullary tissue increased as the result of the further development and expansion of the sinusoids. At 24 hr after irradiation the cellular debris was almost completely removed, the general appearance was that of a node from which the lymphocytes had disappeared, and the sinusoids were dilated and devoid of their lining cells. As the damaged lymphocytes were removed, plasma cells became the most prominent cellular elements. Nodes examined 24 to 36 hr after irradiation showed foci of small lymphocytes reappearing in the cortical areas (Fig. 2.7). The number of these new cells gradually increased, and

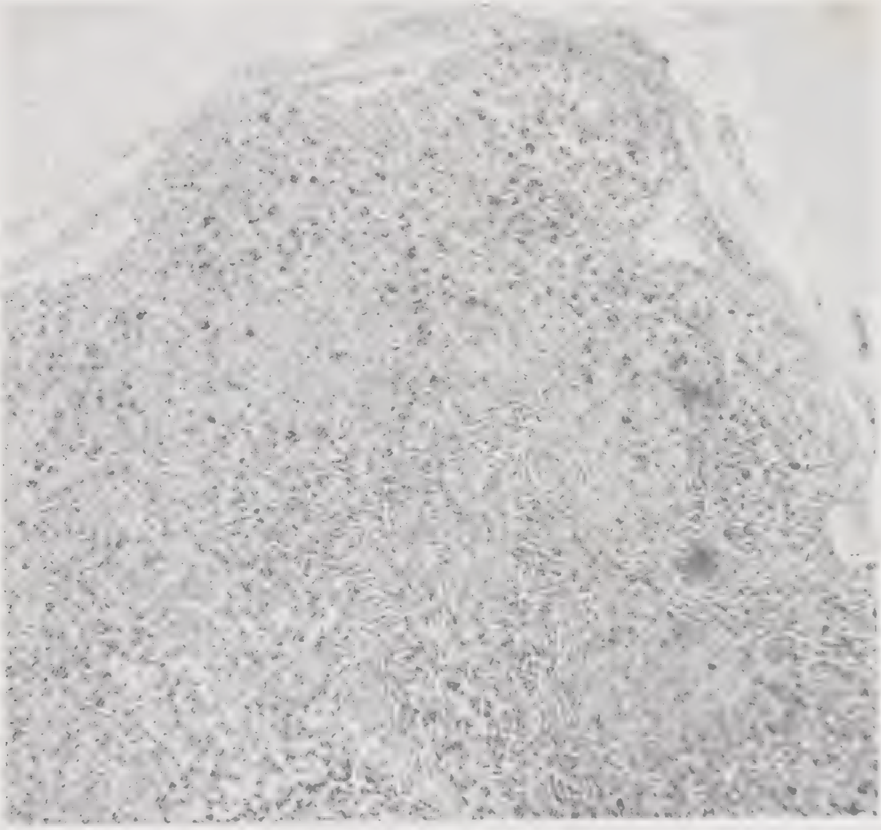

Fig. 2.4—Submaxillary lymph node of a rat removed 2 hr after irradiation. Note the extensive destruction of the cells, especially in the follicles. 100×

by the fourth day definite germinal centers were recognizable. As the number of lymphocytes increased, the cortex gradually thickened and was restored to its normal width and appearance. The lining cells of the sinusoids reappeared at a somewhat slower rate than the lymphocytes in the follicles, but by the twentieth postirradiation day they appeared to have been fully regenerated. Hemorrhagic lymph nodes were seen in some animals between 100 and 300 hr.

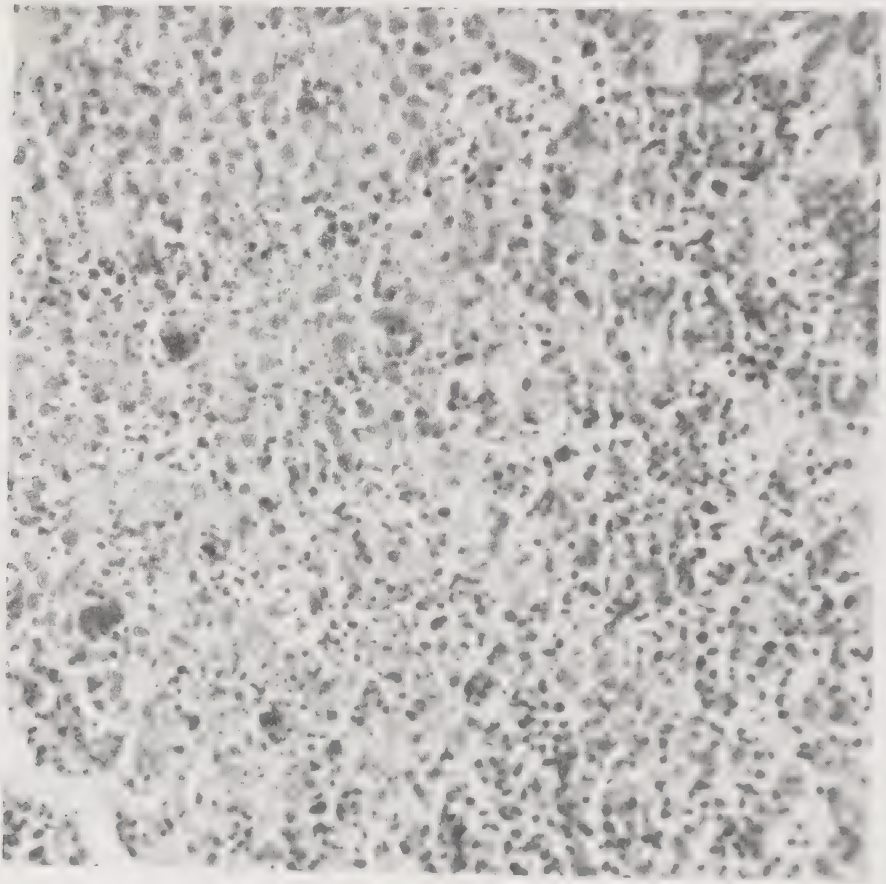

Fig. 2.5—High-power view of one of the follicles in Fig. 2.4. Note the extensive pycnosis and large mononuclear cells engulfing the cellular debris. 430×

3.3 Bone Marrow. As previously pointed out, two methods were employed in studying the changes that occur in the bone marrow following 550 r of whole-body irradiation. A femur and a vertebra from each experimental animal were sectioned and stained in hematoxylin and eosin for a systematic investigation of the reaction of this tissue to irradiation injury. In addition, differential cell counts were made of the marrow smears in an attempt to detect which cellular elements of the marrow are primarily affected and to correlate the changes in the marrow with any alterations appearing in the cell content of the peripheral blood.

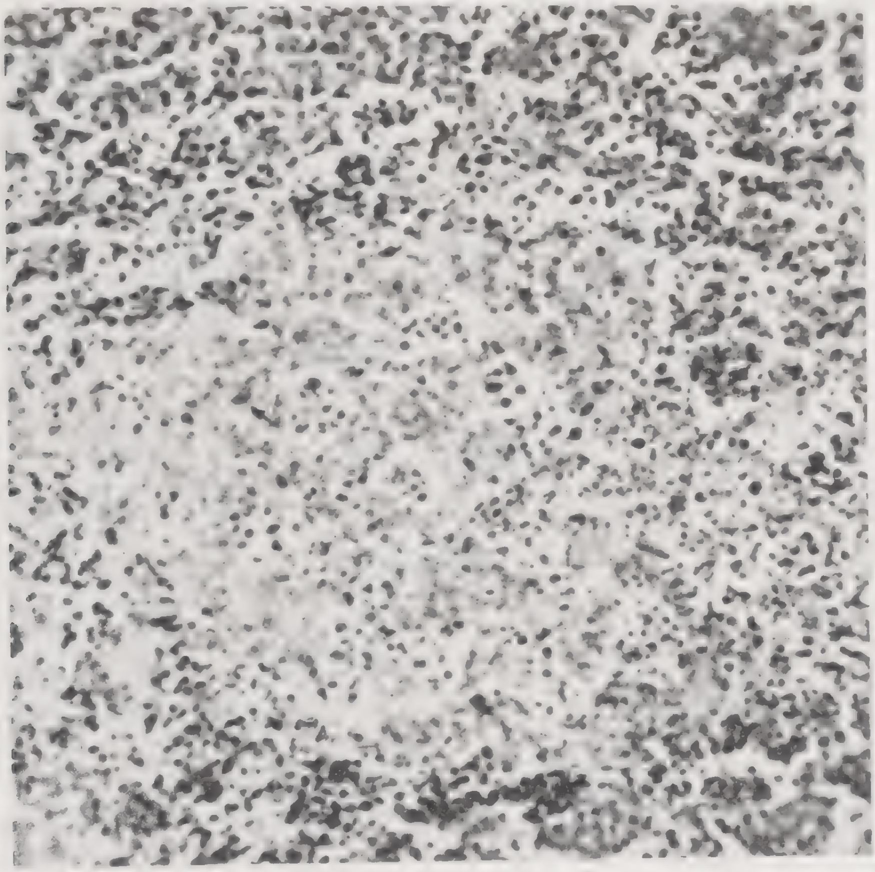

Fig. 2.6—Single follicle of a rat killed 4 hr after irradiation. The number of macrophages has increased, and the size of the follicle is rapidly decreasing. 430×

The histopathological findings in the sections of marrow correspond closely to those previously described for the spleen and lymph nodes. Karyorrhexis and karyolysis were already prominent in the bone marrow of animals killed 15 min after irradiation. Between 2.5 and 5 hr after irradiation, the rate of cellular destruction had reached its maximum. Thereafter, destruction and fragmentation of the marrow cells and phagocytosis of the cellular debris continued at a somewhat less rapid rate. By 30 hr after irradiation most of the accumulated cellular debris had been removed from the marrow. Between 40 hr and 8 days after irradiation, however, there was further reduction in the number of marrow cells so that, by the end of this period, hypoplasia was very severe (Figs. 2.8 and 2.9). Fat cells gradually replaced the parenchymatous cells and eventually became the most prominent cellular element in the marrow. There was no complete aplasia of the marrow in any of the sections studied; representatives of all the cell types were observed.

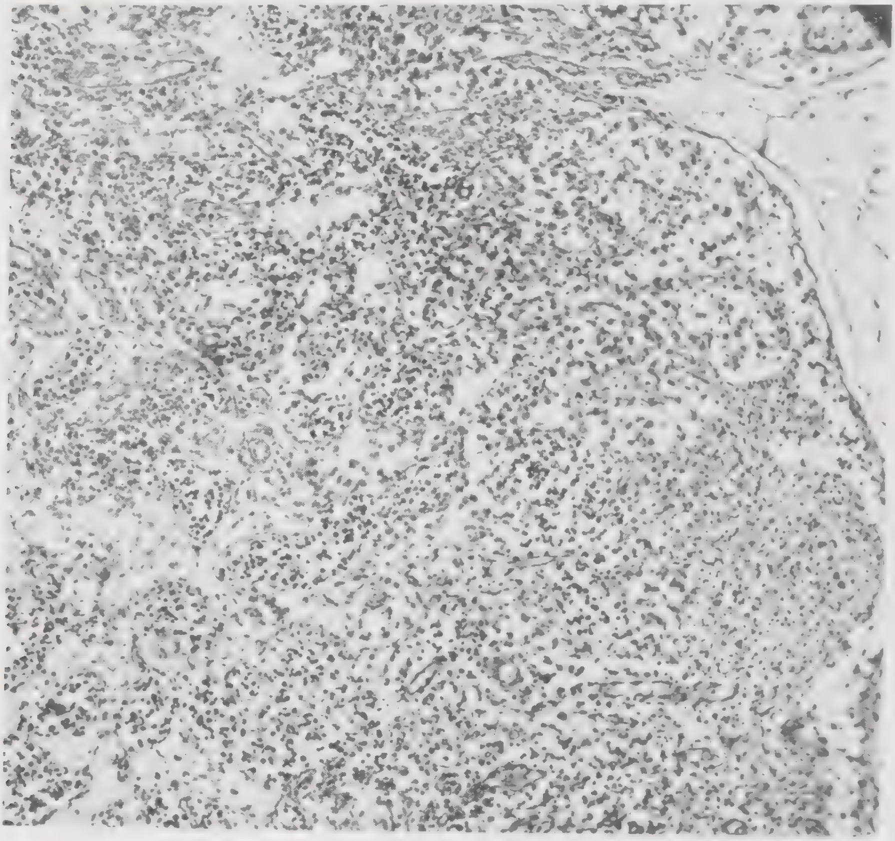

Fig. 2.7 — Portion of a submaxillary lymph node of a rat removed 36 hr after irradiation. Foci of lymphocytes are reappearing in the cortex. The sinusoids are still largely devoid of cells. 100×

In the sectioned material there was some indication of regeneration as early as 6 days after irradiation, but active unequivocal regeneration was not observed until approximately 12 days after exposure.

At approximately 40 days after irradiation, regeneration had progressed to the point at which the irradiated marrows could not be distinguished from the control tissues (Fig. 2.10).

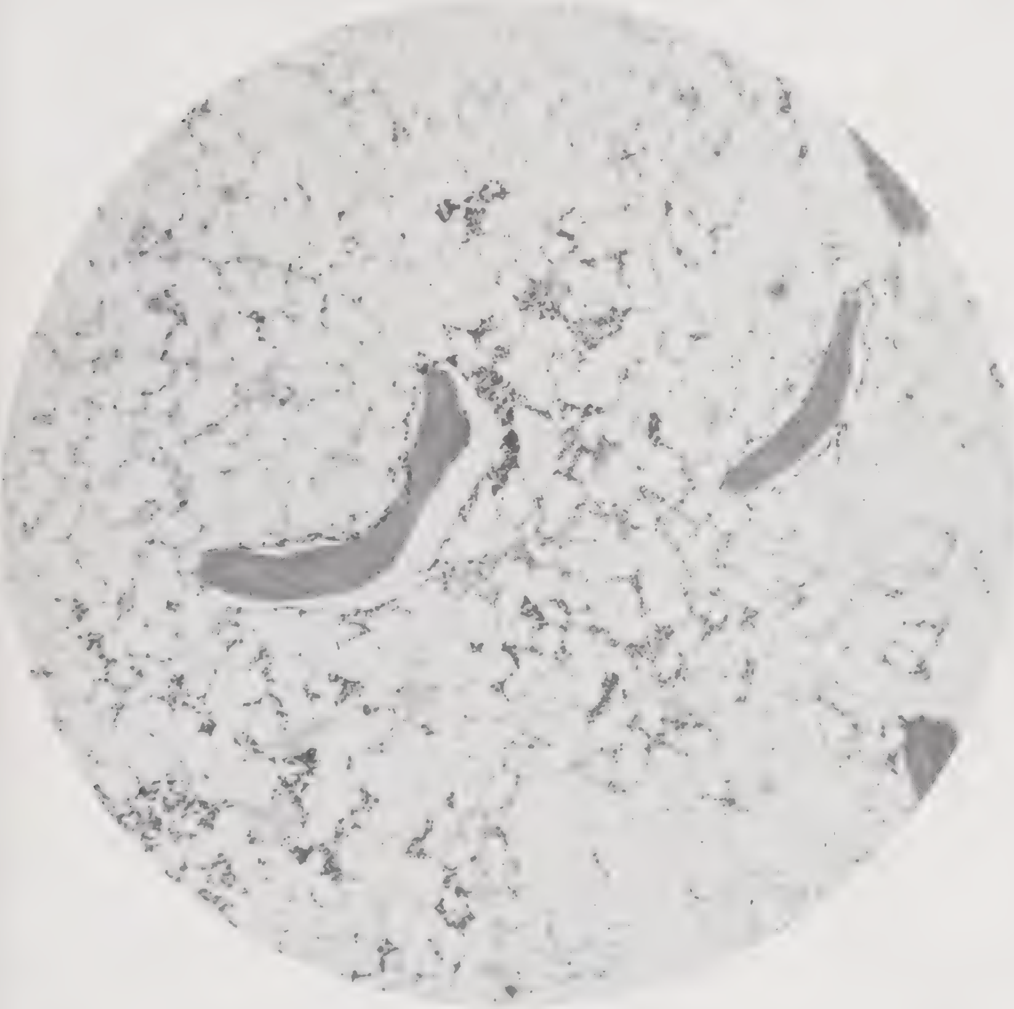

Fig. 2.8 — Bone marrow from the femur of a rat killed 12 days after irradiation. Note the marked hypoplasia and the replacement of marrow cells by fat cells. Foci of bone-marrow cells are still present. 100×

During the period of most marked hypoplasia the sinusoids of the marrow showed considerable congestion, and it could not be determined with accuracy whether, in addition to the congestion, active hemorrhage was occurring.

Differential cell counts were not made on the sectioned marrow, and the only cells receiving attention were the megakaryocytes since they were not included in the differential counts of the bone-marrow smears. In order to detect any change in this cell type, counts were made from the section of femoral marrow, and the results were expressed as the number occurring in 10 high-power fields. These results will be described with those obtained from the study of the marrow smears.

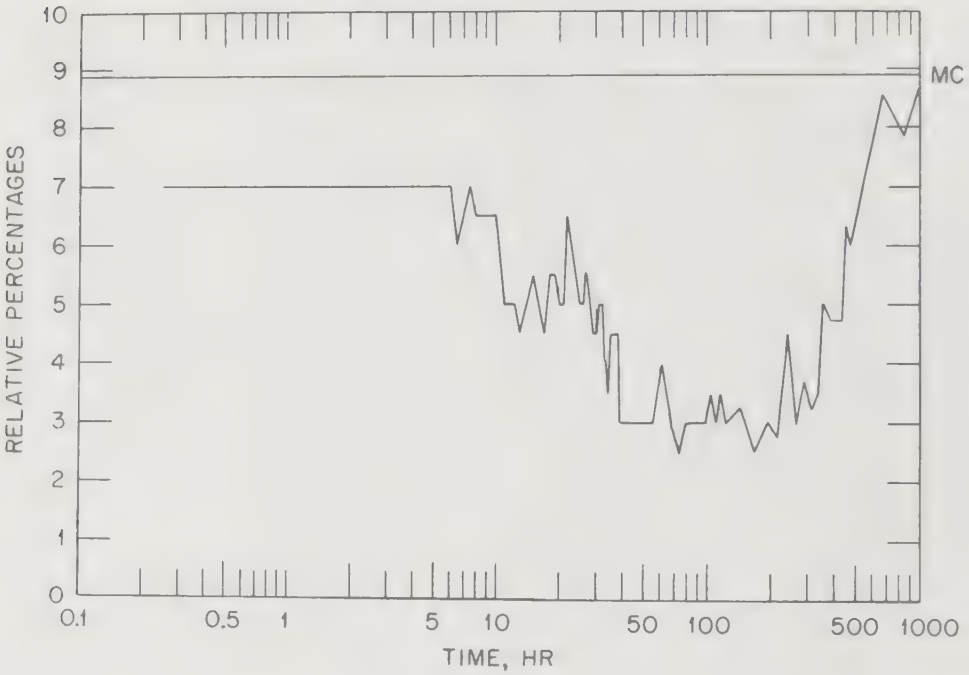

Fig. 2.9—Mean estimate of bone-marrow cellularity.

3.4 Testes. Examination of a single cross section of a normal testis calls attention to the fact that the various seminiferous tubules are in different phases of spermatogenic activity. It is known that for the rat and other animals the generations of cells which succeed one another and which constitute the spermatogenic line have an unequal duration of existence from generation to generation. The result is that in any one location in the seminiferous tubule the epithelium is composed of a number of stages superimposed in order of seniority from the basement membrane to the surface.

In order to evaluate properly the changes observed in the seminiferous tubules of rats irradiated with 550 r, it is necessary to understand the cyclic character of normal spermatogenesis. The normal spermatogenic cycle has been adequately described for the rat by von Ebner (1888) and Regaud (1901), who showed that the various

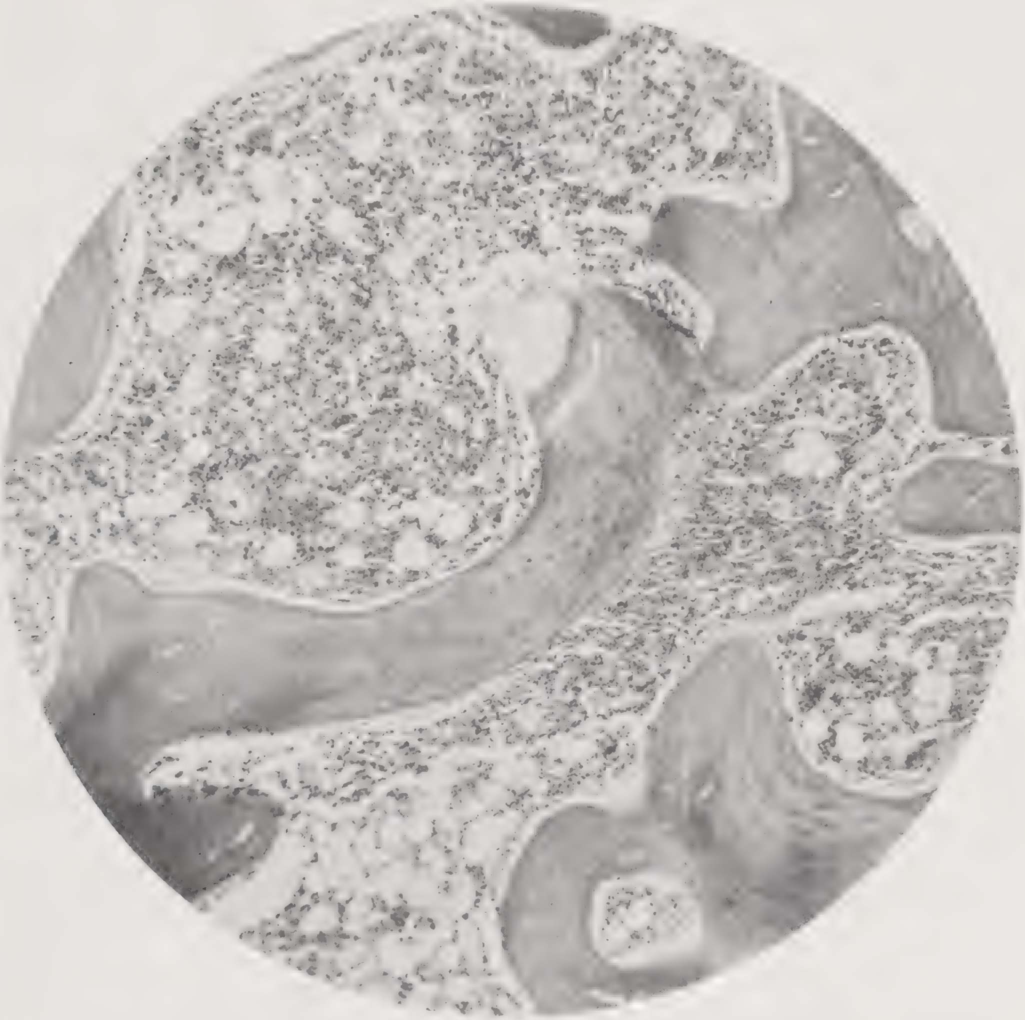

Fig. 2.10—Bone marrow from the femur of a rat killed 41 days after irradiation. The marrow has been completely regenerated and cannot be distinguished from the marrow of the control animals. 100×

cellular combinations of the spermatogenic cycle succeed each other not only in time but also in space, following a more or less spiral course from one end of the seminiferous tubule to the other. The helicoid nature of the spermatogenic movement along the tubule is known as the "spermatogenic wave." The length of a spermatogenic wave has been estimated as 32 mm and requires approximately 20 days for its completion.

Figure 2.11 is a theoretical cross section of a seminiferous tubule, representing the various phases that would be encountered during progression in a spiral course along the tubule. Six distinct phases are recognized: In the first sector, spermatogonia are observed on the basement membrane between the Sertoli cells. In succession, toward the lumen, is a layer of primary spermatocytes and a number

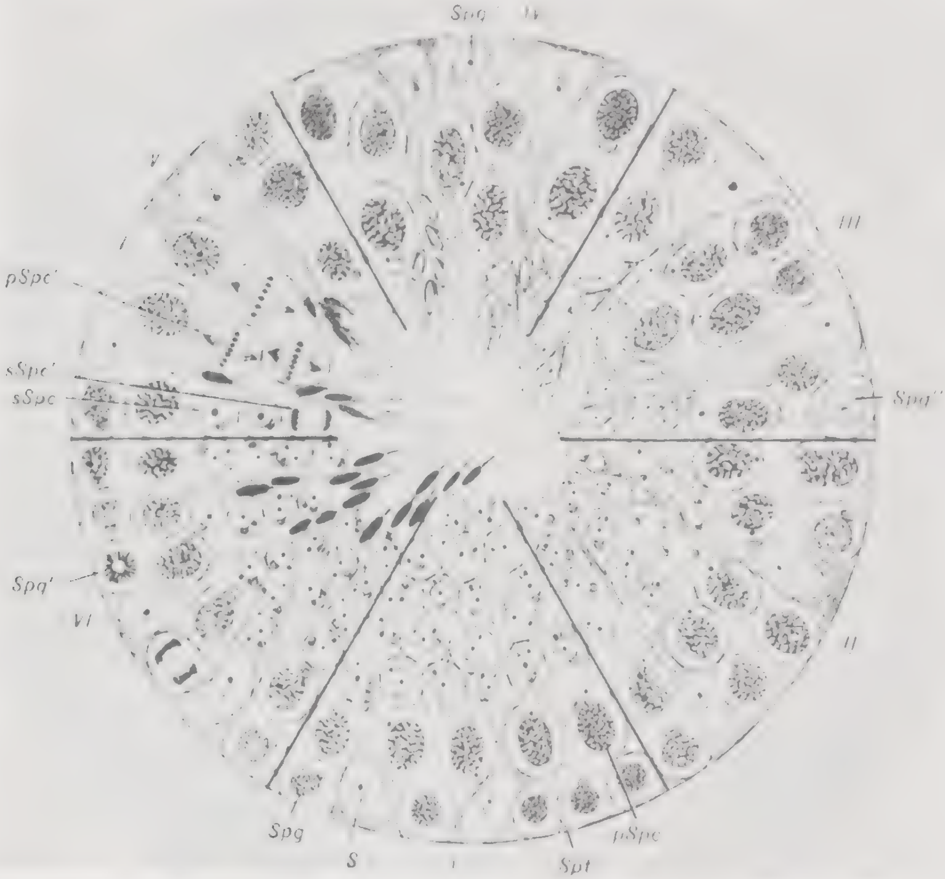

Fig. 2.11—Diagram of six different stages of spermatogenesis. Sectors I to VI indicate the succession of stages as observed in a spermatogenic wave and occupy a spiral position along a seminiferous tubule approximately 32 mm in length. Spg, spermatogonium. S, Sertoli cell. pSpc, secondary spermatocyte. Spt, spermatid (A. A. Maximow and W. Bloom, "Textbook of Histology," W. B. Saunders Company, Philadelphia, 1948).

of layers of spermatids just beginning their transformation. Mature spermatozoa are leaving or have left this segment of the tubule. In the second sector a number of the spermatogonia of the former stage have developed into additional spermatocytes. The spermatids have undergone further maturation and are now closely adherent to the Sertoli-cell cytoplasm. In the third sector a spermatogonium* with a dusty nucleus is seen on the basement membrane. The primary spermafocytes are undergoing their growth phases and can be seen moving nearer the lumen. The spermatids are further advanced in their maturation phases and are still intimately associated with the Sertoli cells. In the fifth sector some of the primary spermatocytes are undergoing maturation, and three mitoses of the first meiotic division are present. The second meiotic division follows rapidly; one diaster is shown (sPpc′). The spermatids have nearly completed their maturation and are now lying nearer the lumen. In the sixth sector, spermatogonia are seen in mitotic division, some of which will form a new generation of primary spermatocytes. The mature spermatozoa are leaving the Sertoli cells and are moving into the lumen beginning their journey toward the epididymis.

In the testes of animals exposed to 550 r the earliest changes are observed in the spermatogonia with crustlike nuclei. By 4 to 6 hr after irradiation a considerable number of spermatogonial nuclei are undergoing karyorrhexis. It is important to note that there is no sudden massive destruction of the spermatogonia. Pycnosis and karyorrhexis may be proceeding in the spermatogonia of one seminiferous tubule, whereas in an adjacent tubule no destruction is yet obvious. Mitotic division in these cells is depressed. The remainder of the cells in the spermatogenic line appear to be developing normally. In tubules in sector V (Fig. 2.11), for example, division of the primary spermatocytes is occurring in the usual manner, and the spermatids are continuing their transformation. At 24 hr after irradiation the destruction of the spermatogonia is still proceeding, and their number is reduced. At approximately 48 hr, tubules may be found in which spermatogonia are completely absent from the basement membrane. In other tubules, destruction of spermatogonia still continues. In some of the animals killed at this time, pycnosis and general disorganization were also occurring in the remaining cells of certain seminiferous tubules. In adjacent tubules, on the other hand, the cells in the spermatogenic line appeared to be normal (Fig. 2.12).

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*Hertwig[6] and others interpret these cells as spermatogonia in an inactive state. They may be found in any sector but ordinarily are not numerous. Spermatogonia with “crustlike” nuclei are more numerous than those with the “dusty” nuclei, and these cells are assumed to be in the early prophase stages of mitosis.

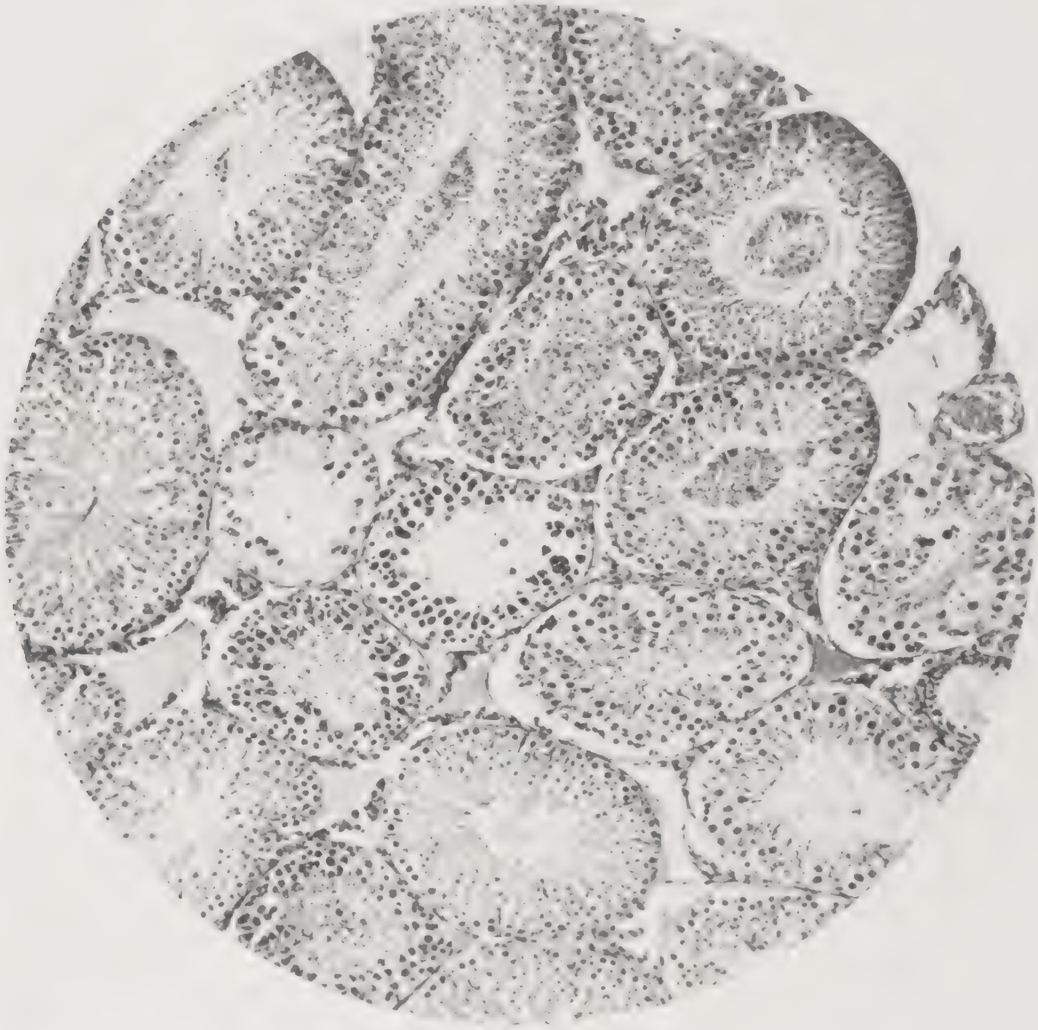

Fig. 2.12—Seminiferous tubules of testis removed approximately 48 hr after irradiation. The extensive pycnosis and disorganization in some of the tubules are obvious. Note that, in adjacent tubules, spermatogenesis appears to be proceeding normally. 100×

By the fourth day after irradiation spermatogonia have largely disappeared from the basement membrane. A small number of abnormal mitotic figures may now be seen among the dividing primary spermatocytes. As the interval after irradiation lengthens and as the cells in the spermatogenic line complete their development, there is a gradual reduction in the total number of cells in the tubules.

At approximately the twentieth day a small number of spermatogonia of the crusty nuclear type again appear in some of the tubules, but mitotic division in these cells is at a minimum.

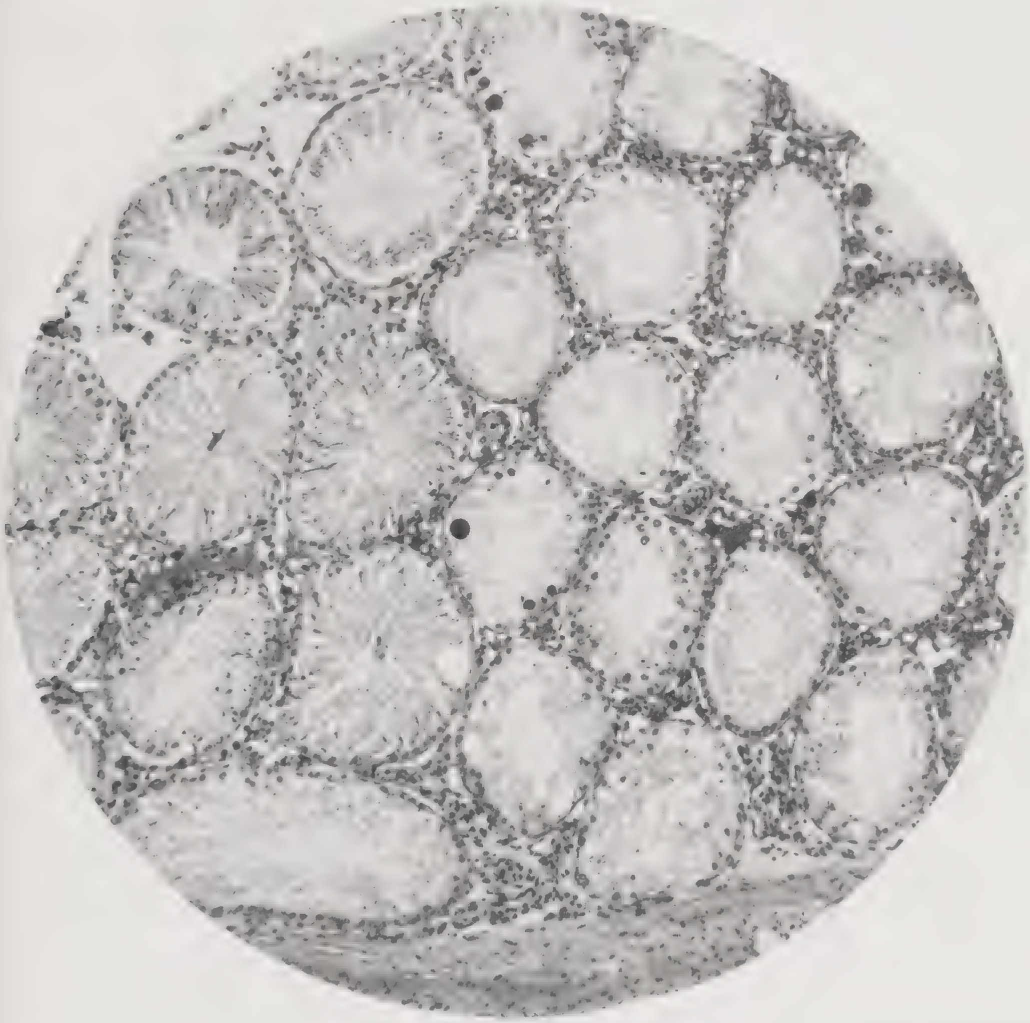

Fig. 2.13—Seminiferous tubules of testis removed approximately 28 days after irradiation. Note the reduction in tubular size and the paucity of germinal cells. Giant cells are numerous. 100×

By the twenty-eighth day the majority of the tubules are completing their spermatogenic cycle, and, since no new cells are being contributed by the basal layer, there is a gradual depletion of cells so that many tubules contain only maturing spermatozoa (Figs. 2.13 and 2.14). Those tubules which have completed the spermatogenic cycle are devoid of cells except for the Sertoli cells and a few scattered spermatogonia lining the basement membrane. Giant cells are frequently found. The general diameter of the tubules is reduced below that of normal.

A larger number of seminiferous tubules are devoid of spermatogenic cells by the thirty-fifth day after irradiation (Fig. 2.15). The spermatids still remaining in the tubules of some of the animals killed at this time showed peripheral clumping of the chromatin, which assumed a more or less signet-ring appearance. A number of these degenerating cells appeared to be aggregating to form giant cells (Fig. 2.16).

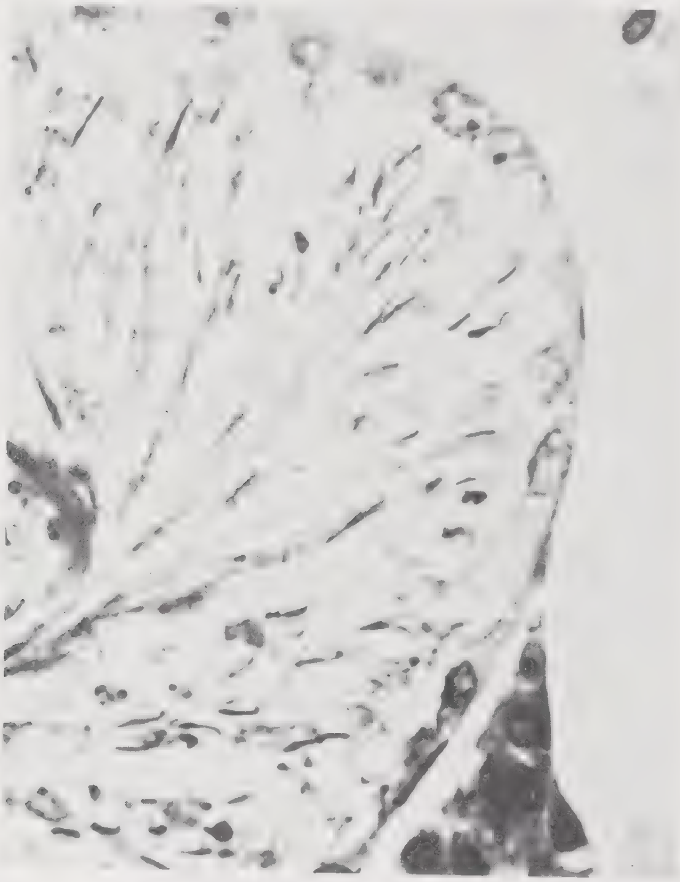

Fig. 2.14—Section of a single seminiferous tubule from an animal killed 28 days after irradiation and showing maturing spermatozoa and Sertoli cells. 600×

On the forty-second day the majority of tubules are lacking in spermatogenic cells (Fig. 2.17), but there is some evidence of the beginning of regeneration. Figure 2.18 is a portion of a seminiferous tubule of Fig. 2.17 in which a number of spermatogonia can be seen in various stages of mitosis. In general, the spermatogonia on the basement membrane are becoming more numerous. In a few tubules a small number of primary spermatocytes are also present. From the over-all picture of the tubules the impression is gained that a considerable period of time must still elapse before any appreciable regeneration would occur. At this time the seminiferous tubules show

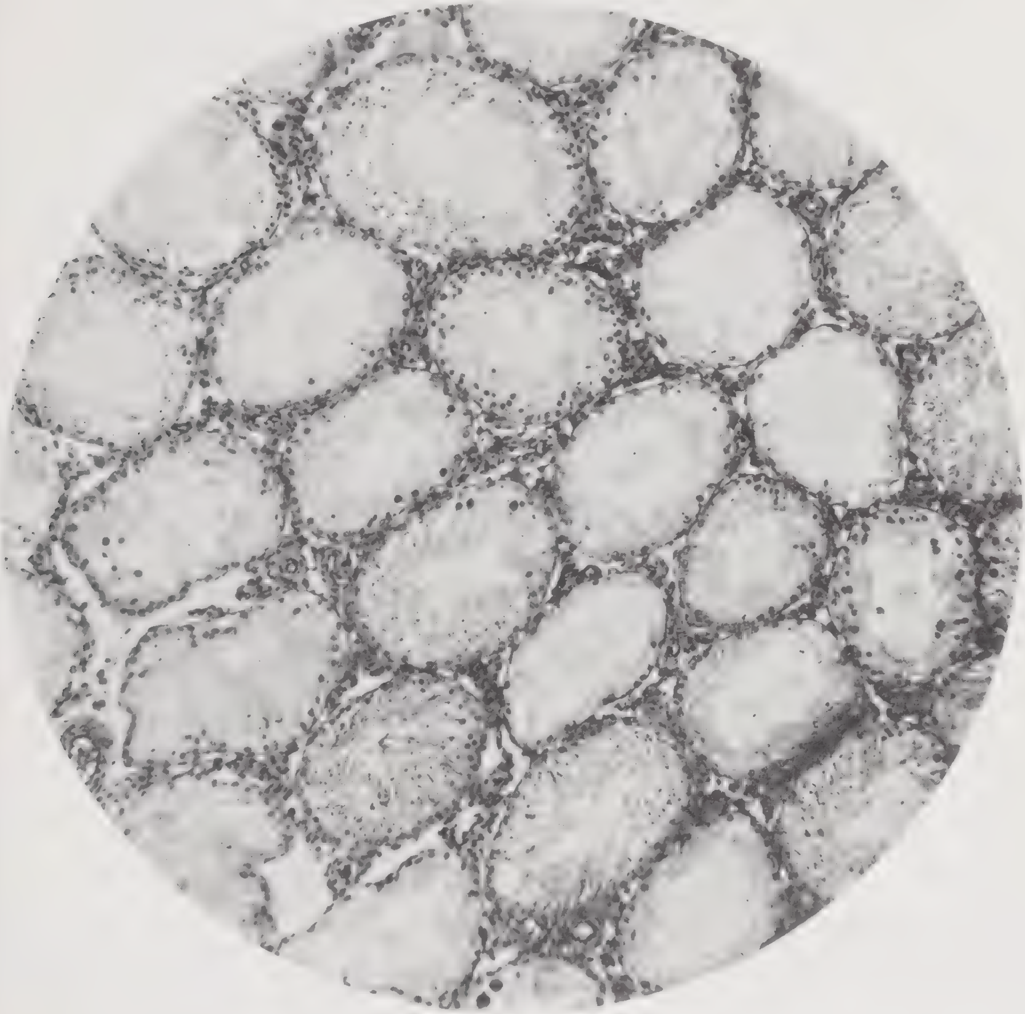

Fig. 2.15—Seminiferous tubules of testis removed approximately 35 days after irradiation. All the germinal cells have disappeared except for a few spermatogonia and primary spermatocytes. The Sertoli cells show no signs of injury. 100×

a distinct reduction in size when compared with the controls (Fig. 2.17).

Between the sixth and seventh days after irradiation, cells in various stages of degeneration appear in the lumina of the proximal parts of the epididymides. Their number gradually increased until approximately the twenty-eighth day, after which it again decreased. Sperm were absent from the proximal epididymides in animals examined between the thirty-fifth and forty-second days. Although greatly reduced in number, sperm were never completely absent from the cauda epididymides.

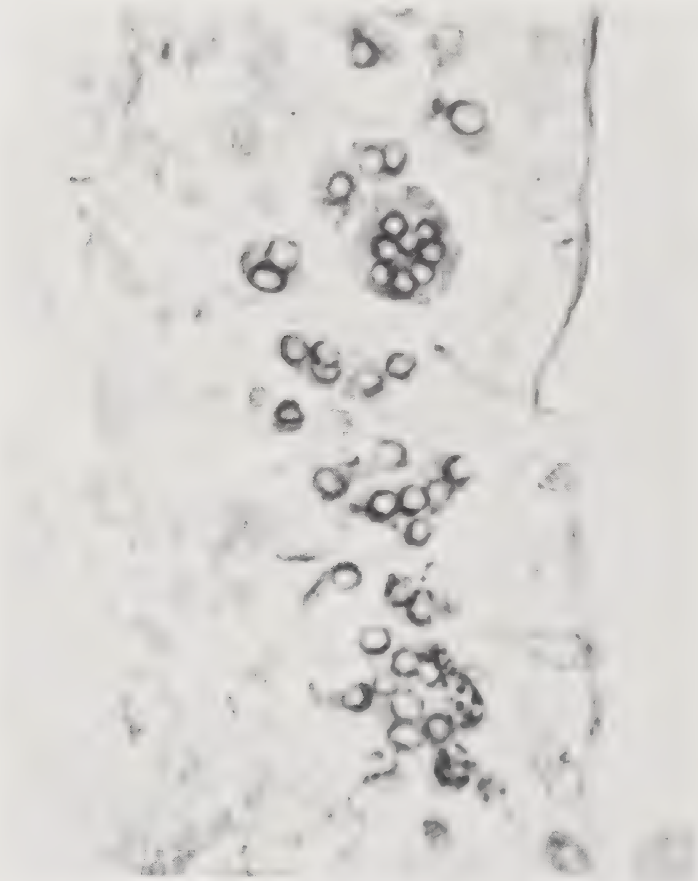

Fig. 2.16 — Part of a seminiferous tubule from an animal killed 35 days after irradiation. Giant cells are forming from the clumping pycnotic spermatids. 600 ×

Sperm removed from the vasa deferentia and examined for motility showed no significant reduction in activity before the twenty-first day after irradiation. Nonmotile sperm were observed in 7 of 23 animals examined between the twenty-first and twenty-eighth postexposure days. In four additional males examined during this period, sperm motility was greatly reduced. Only nonmotile sperm were found in all animals examined between the thirty-fifth and forty-second days after irradiation.

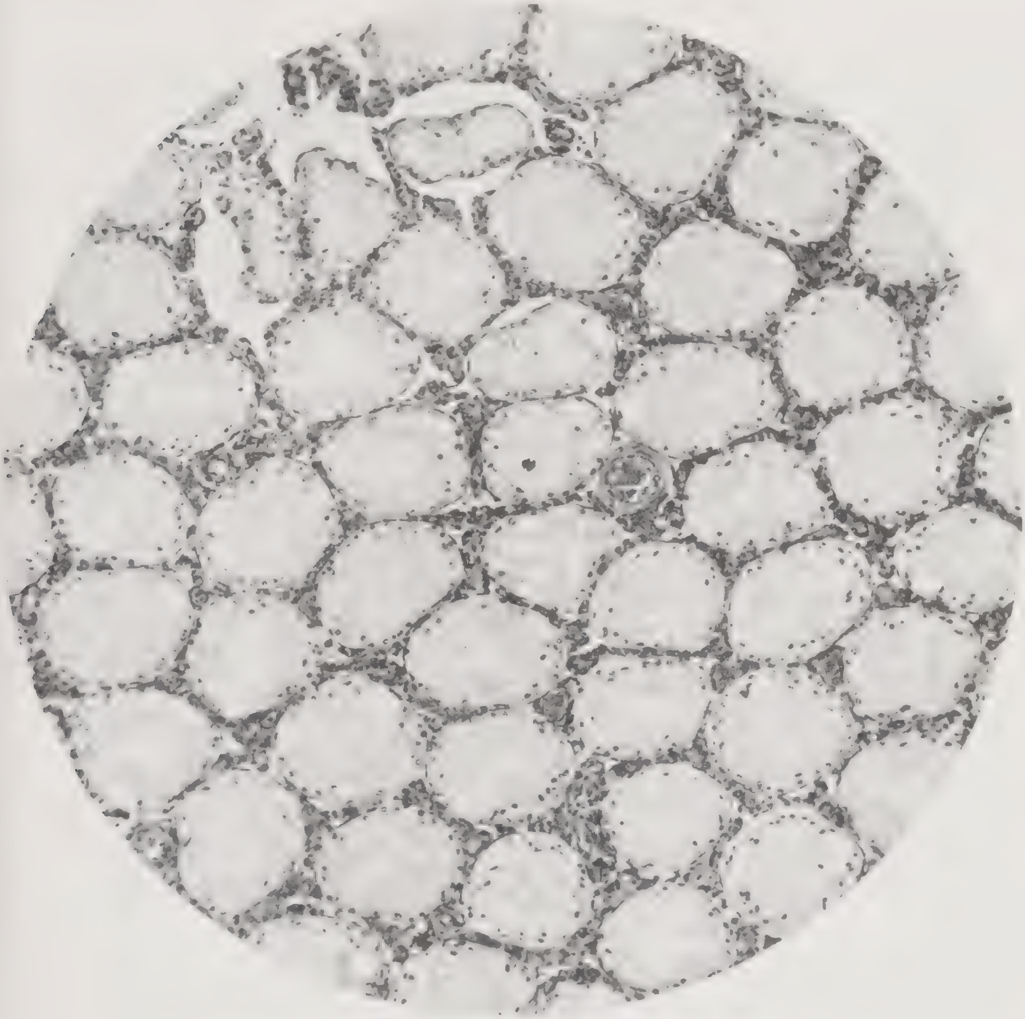

Fig. 2.17—Seminiferous tubules of testis removed approximately 42 days after irradiation. Note the great reduction in tubular size and the relative increase in the amount of interstitial tissue. Spermatogonia are still seen along the basement membrane. 100×

3.5 Ovary. Only two sections of one ovary of each irradiated and control female were prepared for study. Even so, the changes in the follicles that are present in the sections were of such a nature as to warrant description.

As early as 1 hr after irradiation the granulosa cells in the follicles of intermediate size showed pycnosis to a greater extent than in the controls. The number of granulosa cells showing karyorrhexis and karyolysis increased rapidly in the small and intermediate follicles so that by the sixth postexposure hour there was a definite decrease in the number of these cells (Fig. 2.19). In some ovaries, large follicles that were in the preovulatory growth stages were present. In these follicles the granulosa cells did not show as much injury as those in adjacent smaller follicles, and by the study of successive stages the changes occurring during this period of follicular development were apparently proceeding normally. In several females killed 24 to 27 hr after irradiation, recently ovulated eggs still

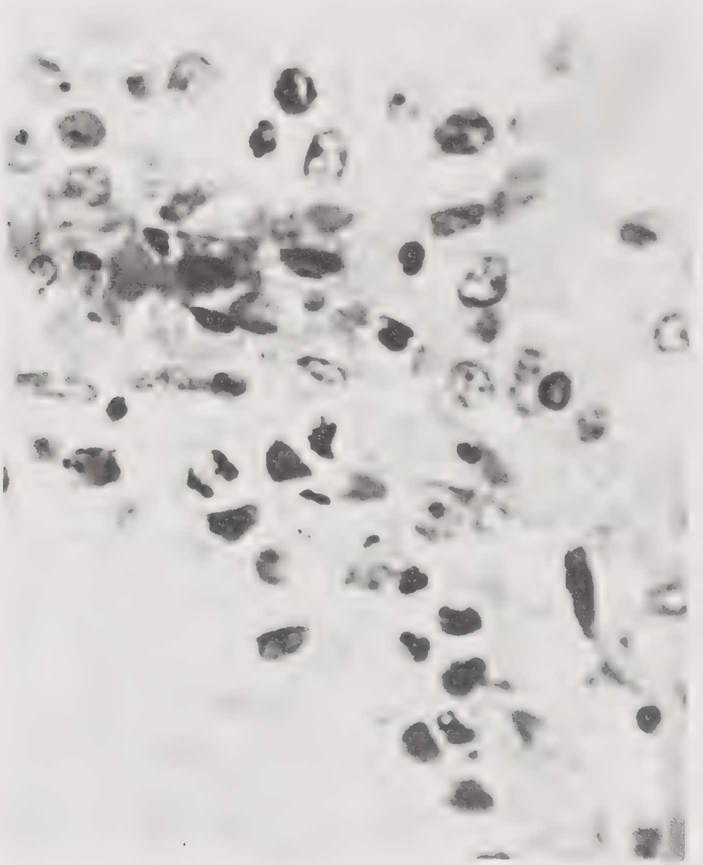

Fig. 2.18—Part of a seminiferous tubule of an animal killed 42 days after irradiation. Several mitotic divisions can be seen in the spermatogonia. 600×

surrounded by their granulosa cells were found in the tubal sacs of the oviduct. As far as could be determined, the ovulated eggs appeared to be normal. The corpora lutea resulting from these ovulations were in the early stages of development and showed no signs of injury or abnormal organization.

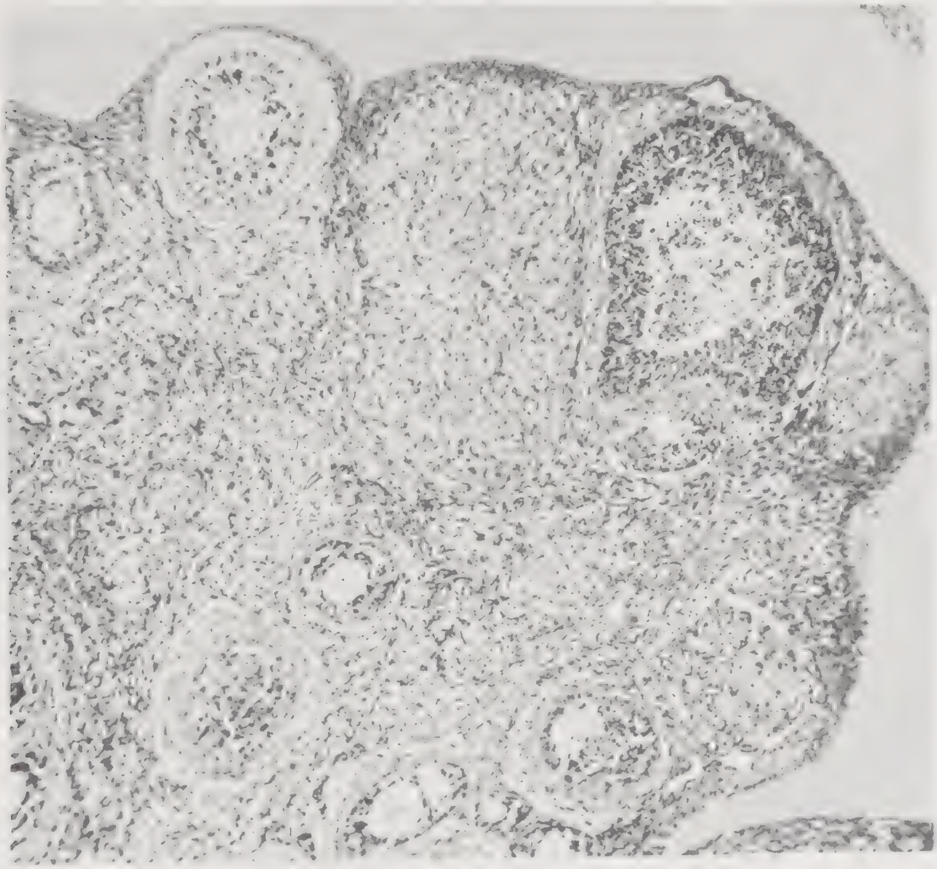

Fig. 2.19—Destruction of the granulosa cells in the intermediate and small follicles in ovaries removed approximately 6 hr after irradiation. 100×

At approximately 20 hr after irradiation the nuclei of the ova of the larger follicles showed definite pycnosis, and their cytoplasm stained more heavily. In the material available, injury could not be conclusively demonstrated in the primary follicles, the theca cells, or the lutein cells of the corpora lutea at any time after irradiation.

3.6 Gastrointestinal Tract. The most striking changes in the gastrointestinal tract were observed in the small intestine, especially in the more proximal portions. Degenerative changes such as karyorrhexis and karyolysis in some of the cells and the swelling of the nuclei in others were already prominent 1 hr after irradiation (Fig. 2.20). The bases of the villi were involved in the degenerative changes to a much greater extent than the tips of the villi.

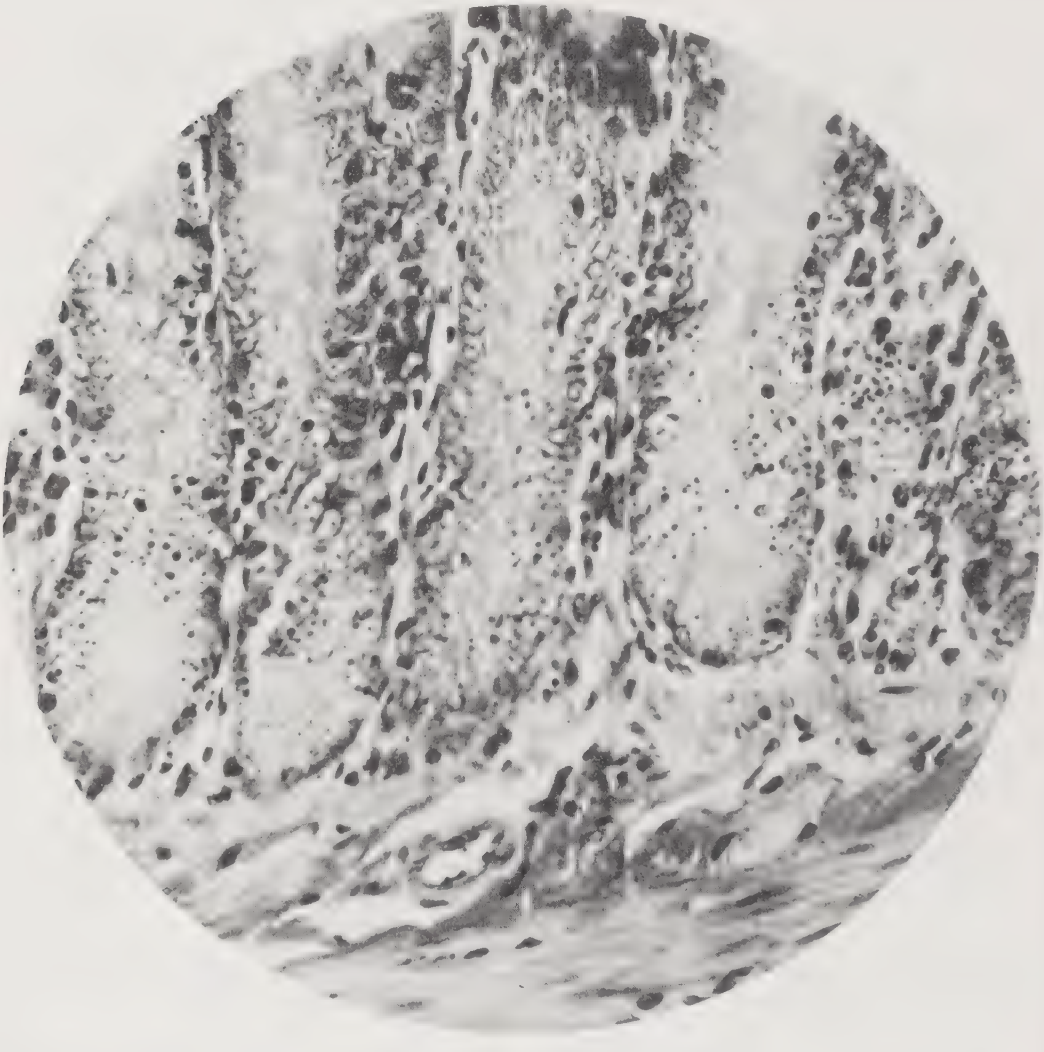

Fig. 2.20—Small intestine removed approximately 1 hr after irradiation. The pycnosis in the basal portions of the glands is prominent. Mitoses are completely depressed. 100×

Another early and striking change that occurred throughout the gastrointestinal tract was the absolute cessation of mitotic divisions, which persisted for approximately 8 hr in the small intestine and as long as 20 hr in the large bowel.

At 2 hr after irradiation, some nuclear pycnosis was observed in the base of the gastric crypts, involving especially the chief cells. These changes were mild and transitory, and no definite cycle of injury and repair could be determined.

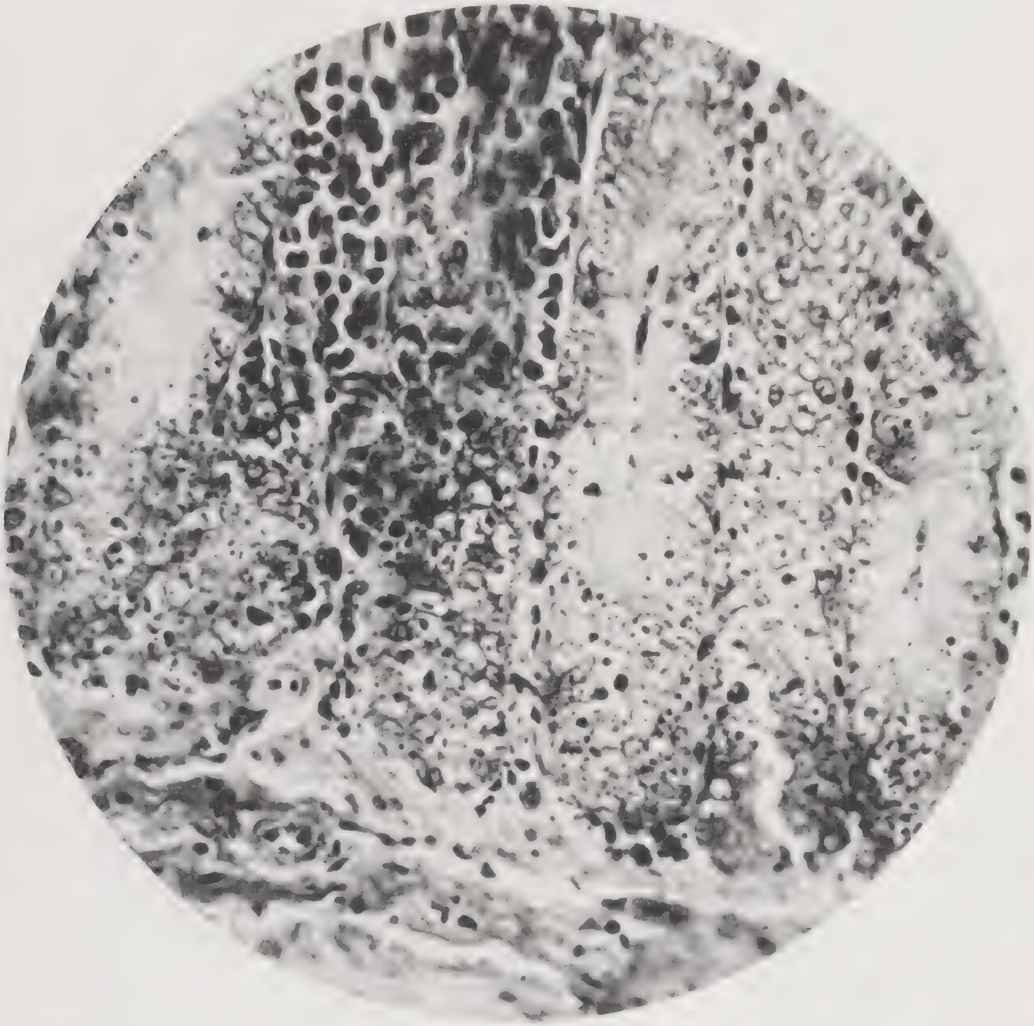

Fig. 2.21—Small intestine removed approximately 4 hr after irradiation. There is a definite increase in the destruction, and the majority of the basal cells are involved. 100×

At 4 hr after irradiation (Figs. 2.21 and 2.22), injury to the basal cells of the small intestine was more evident. Numerous nuclei were undergoing fragmentation. Mononuclear phagocytes were present in large numbers, and many of them were filled with cellular debris.

Along the sides of the villi, pycnotic nuclei were appearing, and mitoses were at a minimum.

In no part of the intestinal tract did injury result in ulceration of the mucosa. The dead cells removed by macrophages were replaced by other cells, often somewhat irregular in shape, which contained prominent and vesicular nuclei. Although the degenerative changes in the bowel were not apparent 30 hr after irradiation (Fig. 2.23), macrophages were still present in the lamina propria. Mitotic figures

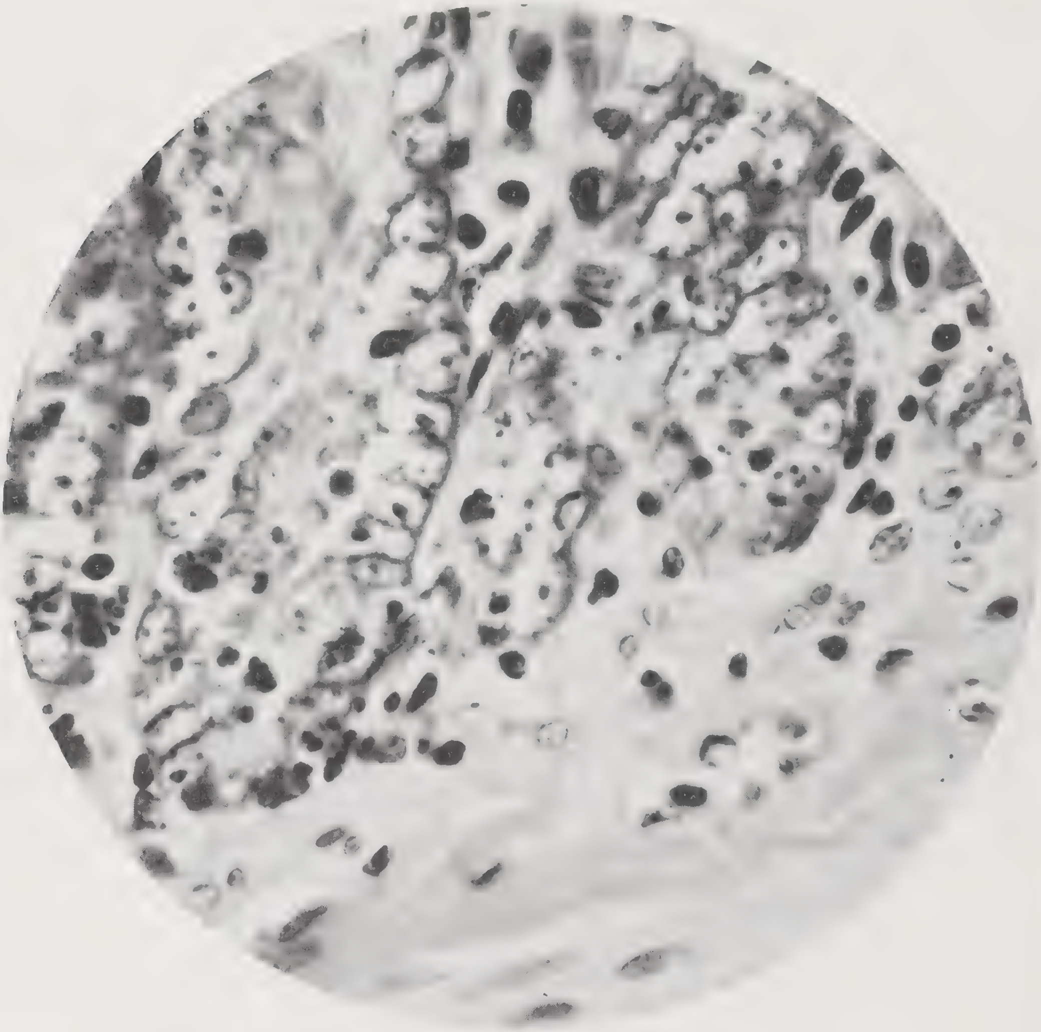

Fig. 2.22—Higher-power view of a part of the intestine shown in Fig. 2.21. Note the large numbers of mononuclear cells engulfing the cellular debris. 950×

had reappeared in moderate numbers at this time, but the number observed still appeared to be less than that seen in the intestinal epithelium of the controls. Between 20 and 60 hr after exposure the most prominent feature of the intestinal mucosa was the presence of

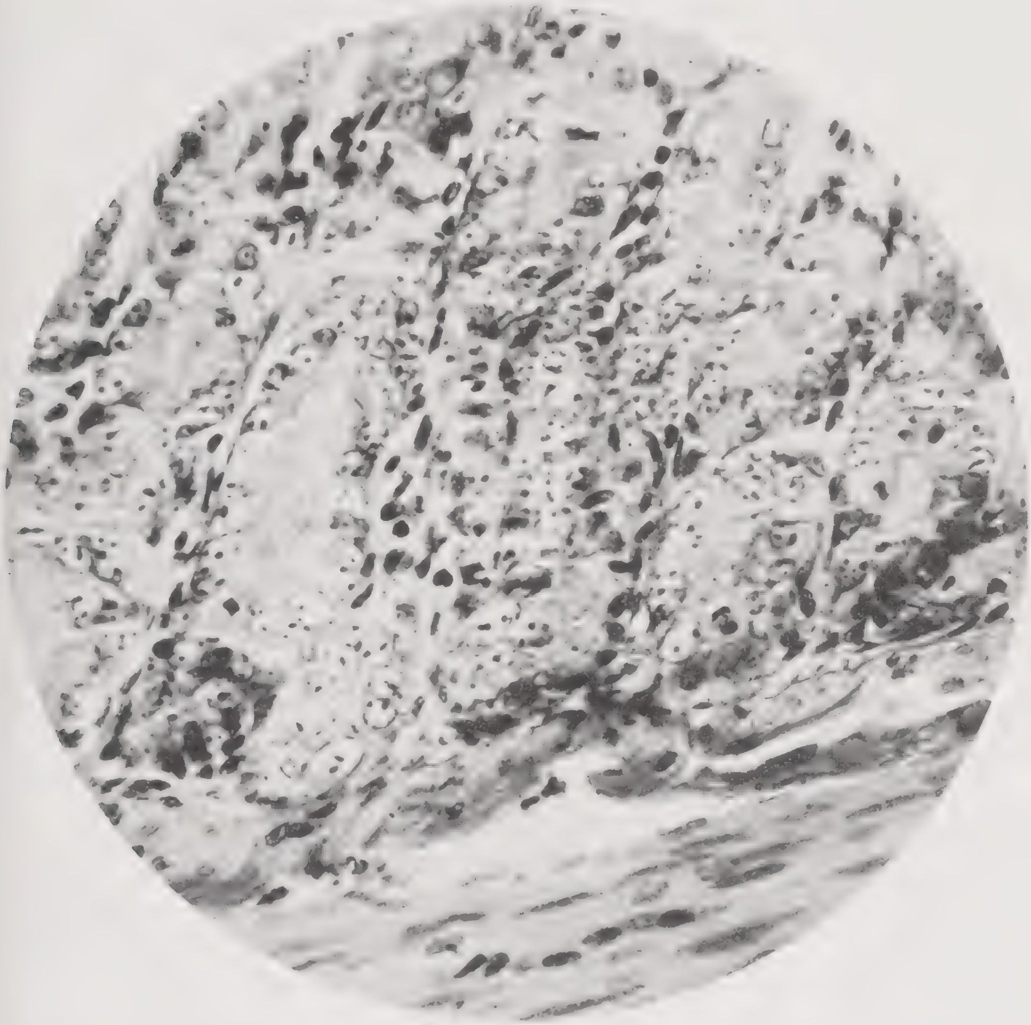

Fig. 2.23—Small intestine removed approximately 24 hr after irradiation. Mitoses are prominent, and regeneration has progressed to a remarkable degree. 100×

the large vesicular epithelial nuclei. The large size and the variations in size and shape that were characteristic of these cells produced a distorted epithelial pattern in the intestinal crypts and along the bases of the villi in contrast to the orderly appearance of the normal mucosa. After 60 hr the appearance of the intestinal mucosa had returned to normal, and the number of mitotic figures was not appreciably different from that in the controls.

3.7 Other Tissues Examined. (a) Kidney. No significant changes were observed in the kidneys of any of the rats in this series.

(b) Lungs. No definite injury to the lung parenchyma could be established in the irradiated animals. The peribronchial lymph nodes present in the sections showed the same sequence of changes described for the submaxillary lymph nodes.

(c) Liver. No definite liver injury could be established in this experiment.

(d) Bone. Examination of the sectioned bones and especially of the epiphyseal plates showed no significant changes when compared with the controls.

(e) Blood and Bone Marrow. As outlined in Sec. 2, the hematological data were obtained from two different groups of animals. In the 180 animals killed at intervals of from 15 min to 41 days after irradiation, peripheral-blood studies were made just before irradiation and again immediately before sacrifice. A second group of 80 animals, given the same amount of irradiation, was divided into smaller groups of 8 animals each. Each smaller group was bled on 6 successive days and then discarded for another group in order to preclude the possibility of anemia due to excessive bleeding. The general trends in the hematological data in these two groups of animals are so similar that they will be combined and described as a single group.

(1) Red Blood Count. There was no significant decrease in the number of red blood cells in the irradiated rats until approximately 100 hr after exposure, when a rapid decline in the number of red blood cells began (Fig. 2.24). The fall in the number of red blood cells continued and reached its lowest level at approximately 15 days after irradiation, when counts of less than 2,000,000 cells/cu mm were made.

From the fifteenth to forty-first days there was a gradual rise in the red blood cell count so that approximately normal levels were reached between the thirty-second and fortieth days after irradiation.

Figure 2.25 is a composite graph of the number and time of appearance of nucleated red blood cells in the peripheral blood. The nucleated red blood cells made their appearance in relatively large numbers during the period of greatest depression of mature red cells. They disappeared from the peripheral blood when the number of red blood cells reached the control levels.

Poikilocytosis, anisocytosis, and basophilia were prominent features of the red blood cells in the peripheral blood at this time. As the number of red cells in the blood returned to normal values, these signs of immaturity of the blood cells disappeared rapidly.

(2) Hemoglobin. The fall in the hemoglobin content of the blood follows relatively closely the decline in the red blood cell count (Fig. 2.24). Values of less than 5 g of hemoglobin per 100 cc of blood were obtained in animals killed between 10 and 16 days after irradiation. After 16 days the rise in the hemoglobin content of the blood increased rapidly, with complete recovery occurring at approximately 32 days after irradiation.

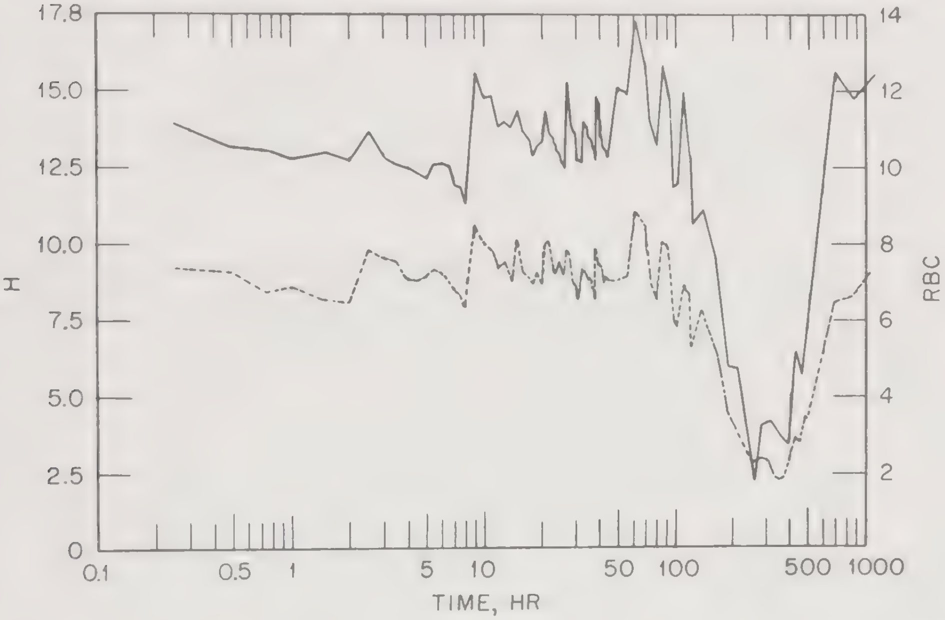

Fig. 2.24—Graph of red blood cell count and hemoglobin content of blood. ---, red blood cell count in millions. ——, hemoglobin content in grams per 100 cc of blood.

(3) Reticulocytes. The number of reticulocytes appearing in the peripheral blood began to increase at about the time of the greatest depression of the red cells in the peripheral blood. The percentage of reticulocytes rose at a rapid rate so that by the twenty-first post-exposure day approximately 40 per cent of the red blood cells were reticulocytes (Fig. 2.25). As the number of red cells in peripheral blood reached normal levels, the reticulocyte count dropped proportionately and reached normal values at approximately 41 days after irradiation.

(4) White Blood Cells. The eosinophils, basophils, and monocytes were present in such small numbers that no definite conclusion could

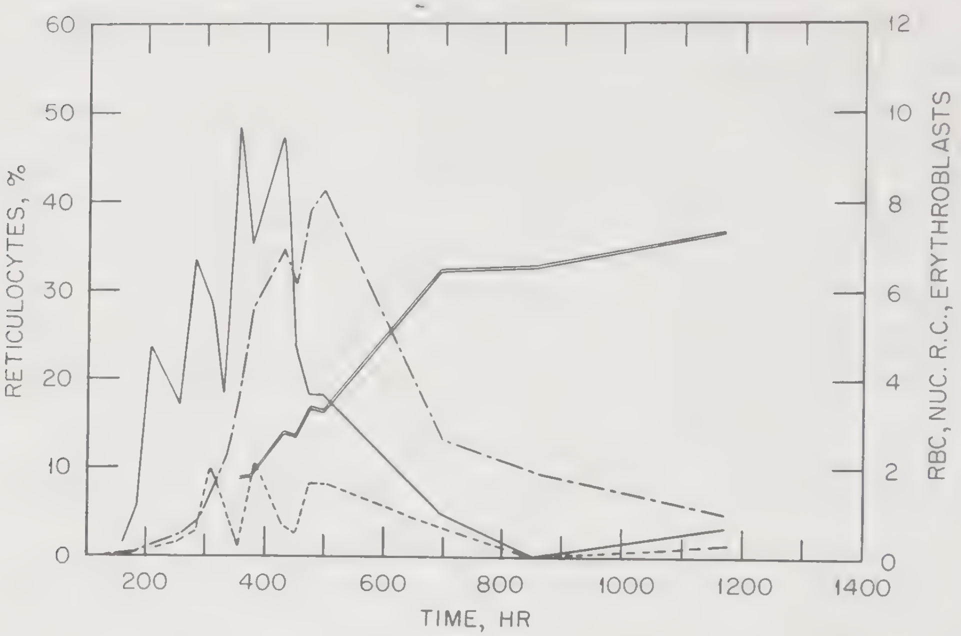

Fig. 2.25—Appearance of precursors of red blood cells in peripheral blood. ═══, red blood cell count in millions. ----, nucleated red cells per 100 white blood cells. ——, erythroblasts per 100 white blood cells. ——-, reticulocytes in percentage.

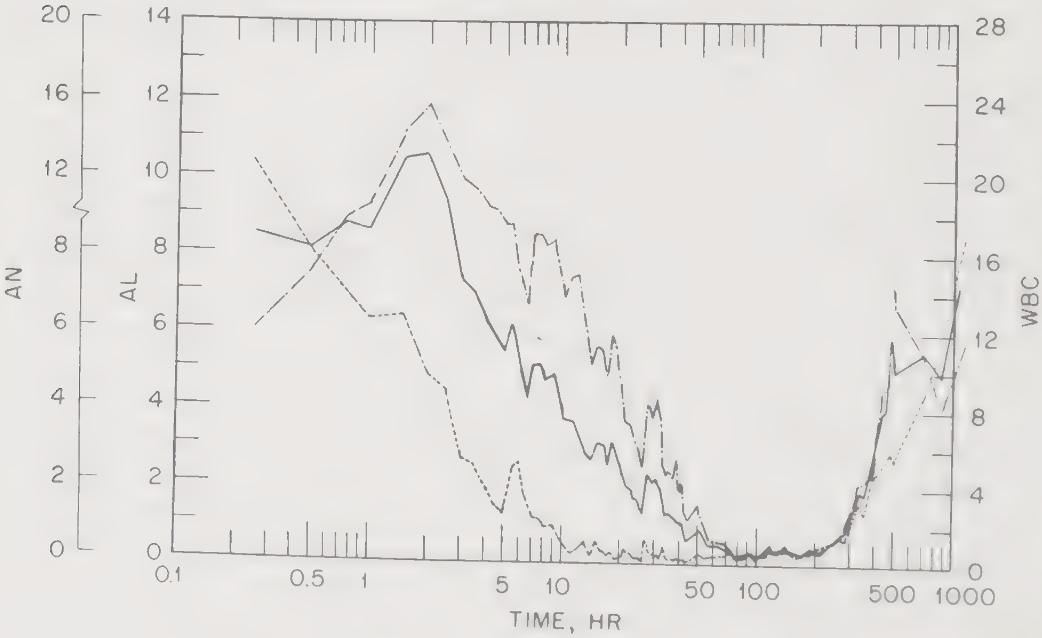

Fig. 2.26—Composite graph illustrating changes that occurred in blood lymphocytes and neutrophils at varying intervals after irradiation. ——, white blood cell count in thousands. ——-, absolute neutrophils in thousands. -----, absolute lymphocytes in thousands.

be drawn concerning the effect of irradiation on them. Figure 2.26 is a composite graph illustrating the striking changes that occurred in the blood lymphocytes and neutrophils at varying intervals after irradiation. The circulating lymphocytes were the first blood cells to undergo reduction. This reduction was present as early as 15 min after irradiation and progressed at a rapid rate so that by the fifteenth postirradiation hour the blood was almost devoid of these cells. The marked depression of blood lymphocytes continued for approximately 10½ days. Thereafter, recovery was slow and was not complete until 41 days after irradiation.

There was a transient leucocytosis during the first few hours after irradiation. This leucocytosis was caused by an increase in the number of circulating neutrophils. Neutropenia began at 12 to 13 hr and continued for approximately 12 days. Recovery of the number of circulating neutrophils then began. The return of the neutrophils was gradual but occurred at a more rapid rate than that of the lymphocytes (Fig. 2.26).

The appearance of the precursors of the white blood cells in the peripheral blood may give a clue to the changes occurring in the bone marrow. A comparison of Figs. 2.27 and 2.9 will show that, when the marrow begins to regenerate and new cells are being formed, the marrow is stimulated to release large numbers of young cells in an attempt to compensate for the deficit of the white blood cells in the peripheral blood. As the mature white blood cells increase in number, the immature cells are rapidly reduced.

(5) Bone-marrow Smears. Figures 2.28 and 2.29 depict the changes in the number of myeloid and erythroid cells in the bone marrow in animals killed at varying intervals after irradiation. The mean value for the controls appears as a solid horizontal line labeled "MC."

In regard to the differential myeloid count of the bone-marrow smears it is interesting to note that there was no marked change in the relative number of these cells for the first 50 hr after irradiation despite the great reduction in total cellularity (Fig. 2.29). Shortly after the second postirradiation day the count of the myeloid elements in the marrow was significantly reduced. This reduction was maintained at a low level from the third to the twenty-eighth postirradiation days. The return of the myeloid cells to the marrow was gradual and reached approximately normal levels at the forty-first postexposure day.

In the erythroid series a depression in the cell count was observed at a considerably earlier period (Fig. 2.28). At 6 hr after irradiation the relative number of erythroid elements had already been significantly reduced. This depression continued for approximately 5½ days, after which there was a rapid return to normal levels. As would be expected, the counts made of the neutrophils and eosinophils in the marrow smears corresponded closely with those of the total myeloid cells, and the graphs on the whole are very similar (Figs. 2.30 and 2.31).

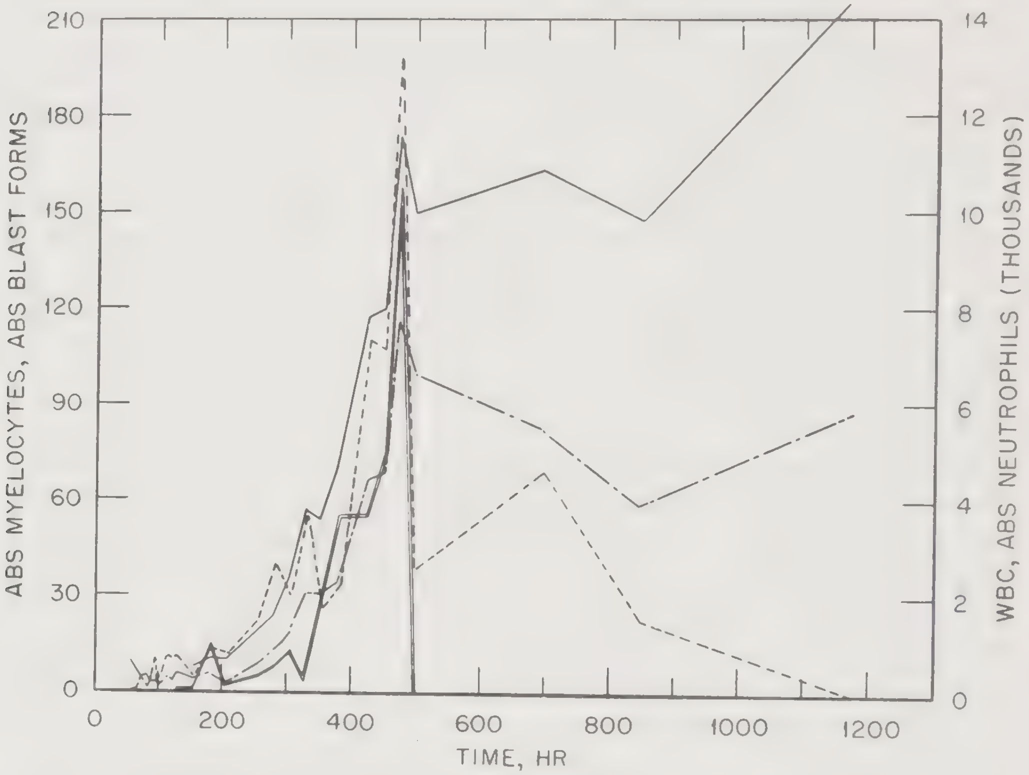

Fig. 2.27—Appearance of precursors of white blood cells in peripheral blood. ——, white blood count. —–-, absolute neutrophils. ═══, absolute myelocytes. ----, absolute blast forms.

As the marrow became hypoplastic the basophils did not vary significantly from those in the controls except for the reduction in the absolute numbers.

(6) Megakaryocytes and Platelets. Megakaryocytes were counted in the femoral marrow of animals killed at varying intervals after irradiation, and the results are presented graphically in Fig. 2.32. In animals killed during the first 3 hr after irradiation, megakaryocytes were present in reduced numbers when compared to the controls. The greatest reduction in the number of these cells occurred between 4 and 13 days after irradiation. This period corresponds approximately to the period of greatest hypoplasia as observed in the sectioned marrow. Even at 41 days after irradiation the number of

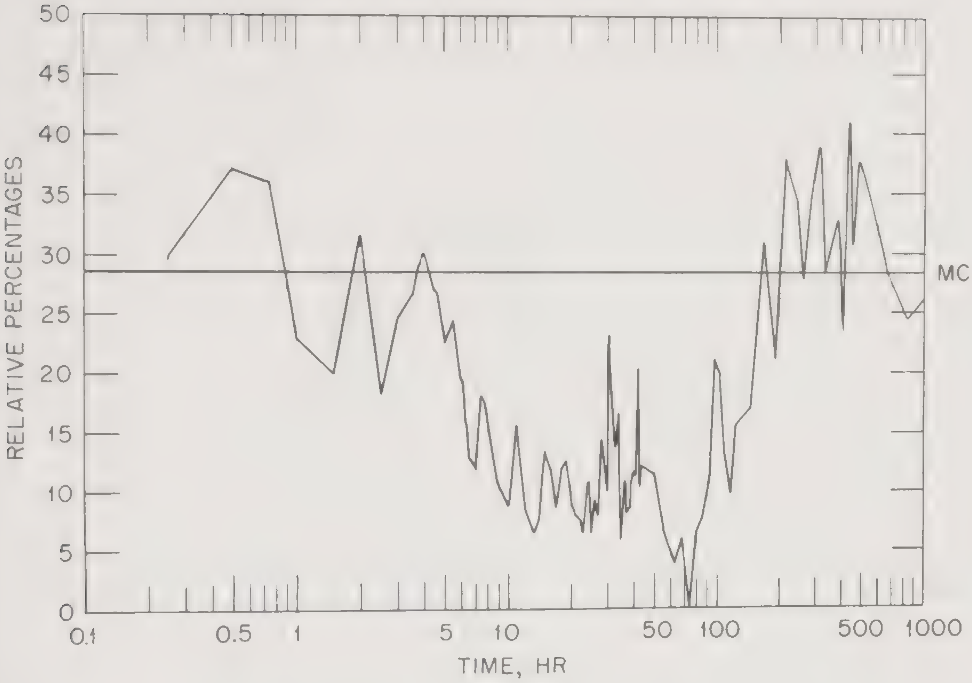

Fig. 2.28 — Mean of total erythroid count in bone marrow.

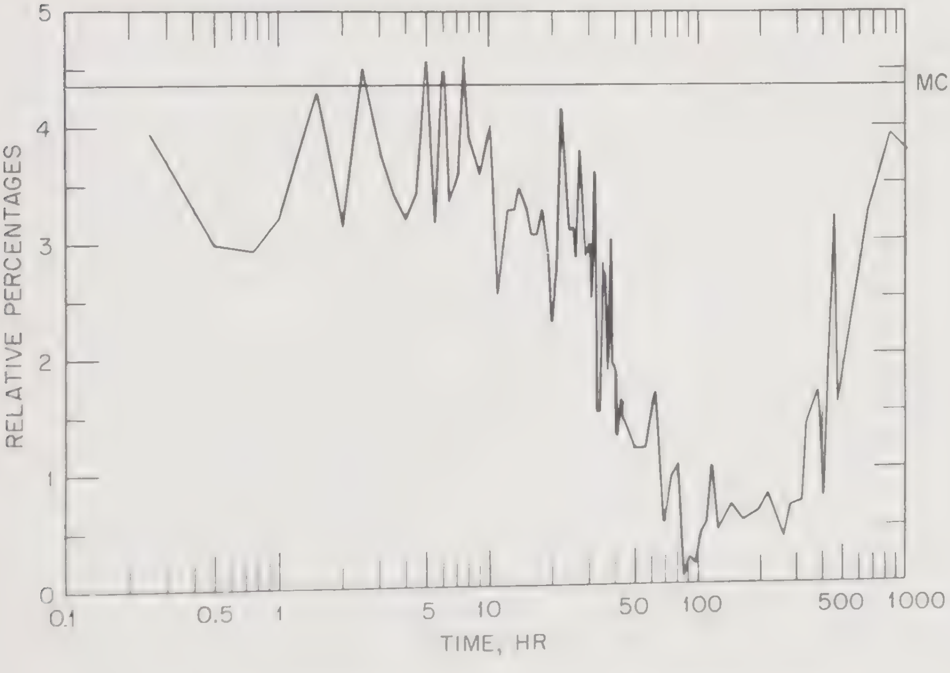

Fig. 2.29 — Mean of total myeloid count in bone marrow.

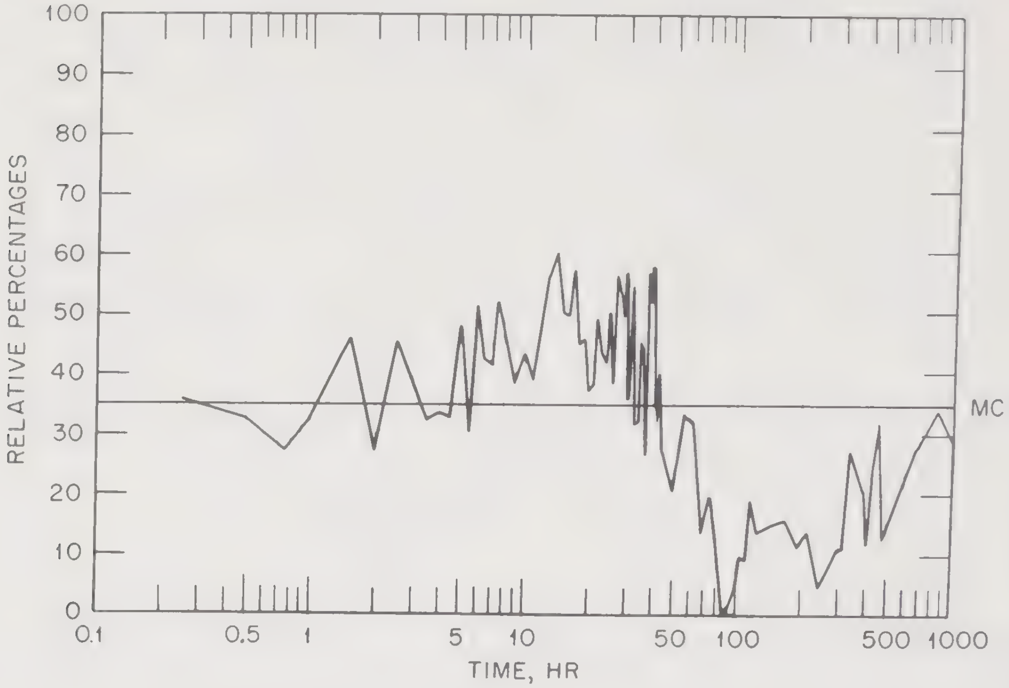

Fig. 2.30—Mean of neutrophils of bone marrow.

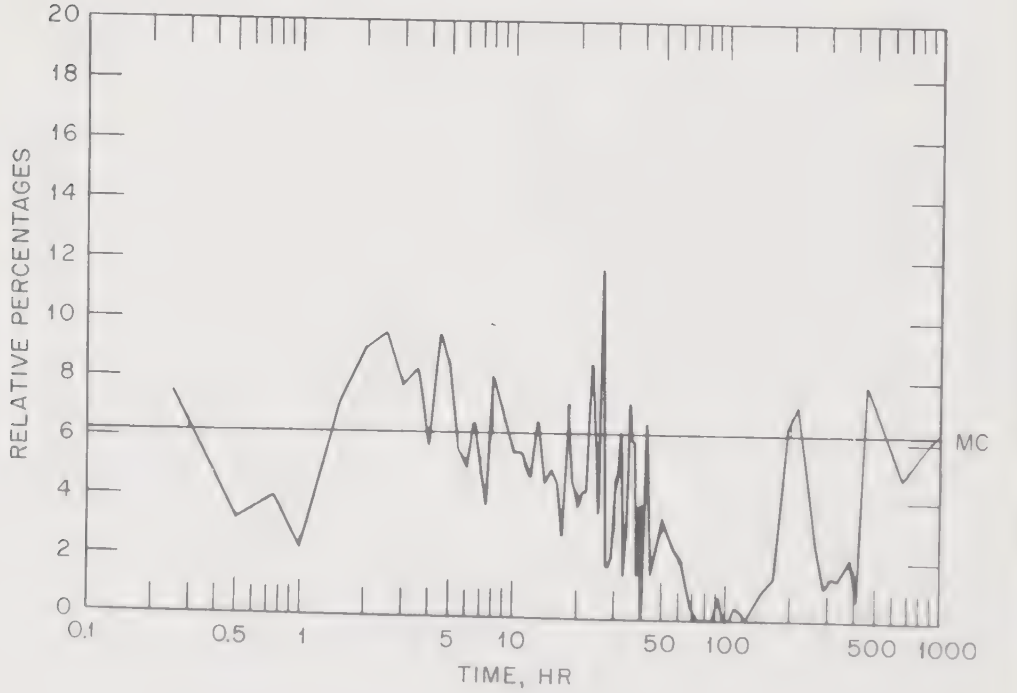

Fig. 2.31—Mean of eosinophils of bone marrow.

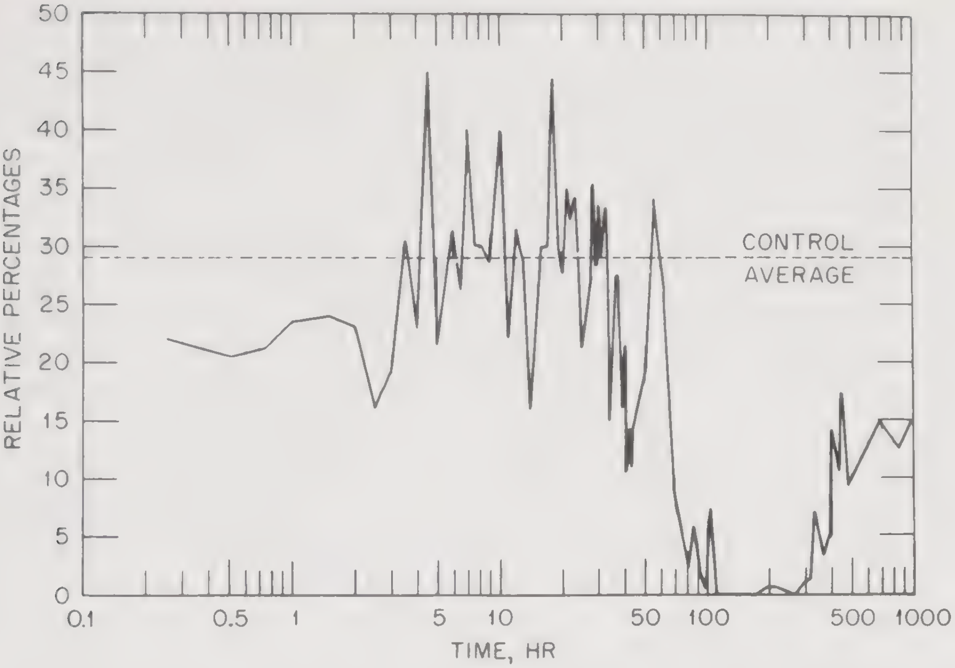

Fig. 2.32—Megakaryocytes in split femur section (average number in 10 fields).

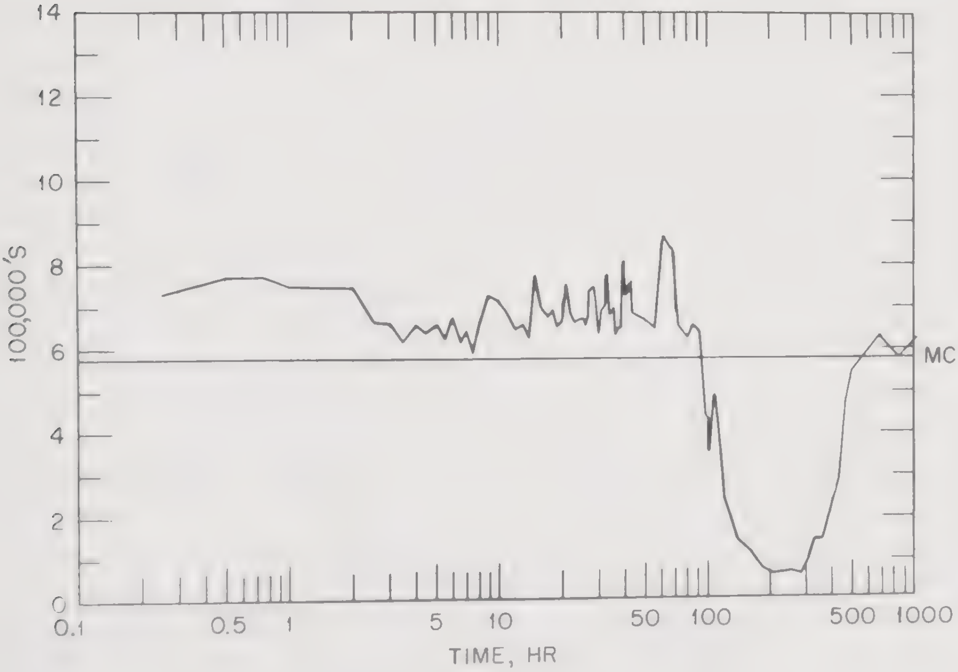

Fig. 2.33—Mean of platelets in peripheral blood.

megakaryocytes present in sectioned marrow was not so great as in the controls.

Of interest is the corresponding graph, Fig. 2.33, which shows the platelet count of the peripheral blood. The most active depression of the platelets occurred at about the time of the greatest depression of the megakaryocytes and of the most extensive hypoplasia of the marrow. Recovery of platelets in the peripheral blood began at about 12½ days and reached normal values at about 41 days.

(7) Animals That Died After 550 r. A total of 86, approximately 25 per cent, of the experimental animals died spontaneously. No animals died prior to the sixth postirradiation day, and the greatest number, 67, died between 6 and 21 days after irradiation, which is the approximate period of the greatest marrow hypoplasia. From the autopsy records and tissue examinations, the primary cause of death in the majority of animals was probably generalized septicemia.

4. DISCUSSION

Examination of the experimental results revealed that in the rat a single whole-body exposure to 550 r of irradiation tremendously disturbs many of the tissues of the body, whereas other tissues are affected to a lesser degree or not at all.

The authors agree with the majority of other investigators[1,7,8] that lymphocytes are the most radiosensitive cells in the body and that a drop in the peripheral lymphocyte count is the earliest and most consistent blood change. The lymphocyte count in the peripheral blood does not always accurately reflect the state of damage or repair in the lymphatic tissue. Although the decline in the number of lymphocytes in the blood follows closely the histopathological changes observed in the lymphatic tissues, the return to normal progresses at a much more rapid rate in the lymph nodes and spleen than in the circulating blood. The lymphatic tissue is not only highly sensitive to irradiation but also has a remarkable capacity to recover rapidly from extensive damage, at least as far as morphological repair is concerned.

Polymorphonuclear leucocytes are considerably less sensitive to irradiation than the lymphocytes. The characteristic response in both man and experimental animals to relatively large doses of irradiation consists in an initial brief leucopenia followed by a leucocytosis that persists for a few hours. Following this, there occurs a more marked and prolonged leucopenia. From our studies it appears that the transient leucocytosis following total-body X irradiation is a result of a temporary increase in the neutrophils. Dunlap,[9] by a careful determination of the absolute values of lymphocytes and neutrophils, shows that this initial rise in the neutrophilic cells is an expression of an initial stimulation of the bone marrow. This does not seem plausible in the light of our experience. One of the earliest and most sensitive reactions of viable cells is a suppression of their mitotic rate. More intensive irradiation produces definite histogenetic abnormalities or complete disintegration of the cells. Although our experiments lack the necessary proof, it would seem more logical to explain the initial but brief rise in the circulating neutrophils as a mobilization of mature cells into the general circulation or to an increased maturation rate of young cells that were near the end of their development cycle at the time of irradiation.

In our experiments the absolute number of neutrophils increased considerably during the first 2 hr after irradiation. Then the number dropped rapidly until approximately 12 hr, when neutropenia supervened and reached its lowest level between the second and eighth days. Comparison of the graph of the estimate of cellularity of the marrow with that of the absolute neutrophil count shows that the decrease in the neutrophils in the peripheral blood is closely correlated with the progression of hypoplasia in the marrow.

Dunlap[9] points out that, although appreciable changes are occasionally seen in the numbers of eosinophils, basophils, and monocytes in the peripheral blood of irradiated animals, these changes are not regular or predictable. Piney and Mayneord[10] did not observe changes in the number of monocytes in the peripheral blood in their irradiated animals. On the other hand, Wünsche[11] described a monocytosis during the period of marrow repair. In our experimental animals no significant changes were noted in the basophils at any time. The number of eosinophils, however, fell to low levels during the period of greatest marrow hypoplasia. Monocytes increased appreciably in the peripheral blood during the period of active bone-marrow regeneration.

The literature contains only fragmentary information concerning the behavior of platelets after irradiation. It has been observed that heavy irradiation of wide areas of the body can result in such a profound drop in the number of platelets that purpura and hemorrhage result.[12] Helber and Linser[13] found a decided decrease in the number of circulating platelets in small animals following heavy irradiation. In our experiments the close correlation between the drop in the number of platelets and the decrease in the number of marrow megakaryocytes is very striking. Of interest in this connection are the observations of Denstad[12] in man, in which he found that the megakaryocytes disappeared from the marrow almost simultaneously with the youngest myeloid cells. In our experiments there was also a close correlation between the reduction in the number of megakaryocytes and the disappearance of myeloid cells in the marrow. The platelet count in the peripheral blood increases in direct proportion to the reappearance of the megakaryocytes in the marrow.

Although gross hemorrhage was infrequent, frequency of microscopic hemorrhage in the bone marrow from 50 to 120 hr and to a lesser extent in the lymph nodes from 150 to 400 hr was noted. The period of greatest frequency roughly paralleled the interval of greatest platelet reduction. The hemorrhages were never severe and could not be considered the primary cause of death in any of the animals.

In man, dog, and monkey the life span of the red blood cell has been estimated to be approximately 100 days.[14–17] If this is assumed to be true in the rat, it is necessary to explain the precipitous fall in the circulating red blood cells that occurs shortly after 100 hr. There is a similar reduction in the platelets beginning around 90 hr and reaching a maximum at 200 hr. There is a fair correlation of platelet reduction, hemorrhage, and low red blood cell count near the 100-hr period. It is believed that the hemorrhagic tendency, at least in part, accounts for the marked reduction in red blood cell level at this period. Our findings do not support the hypothesis that the destruction of the red blood cells by phagocytosis is greater than would be expected in soft-tissue hemorrhage.

Our experiments confirm the observation of Minot and Spurling[7] that the circulating red blood cells decrease in number at a considerably later period than the leucocytes since the fall in red blood cells did not begin until approximately 100 hr after the onset of reduction in the white blood cells. A reduction in the hemoglobin was observed concomitant with the decrease in the erythrocytes in the peripheral blood.

It is of interest to compare the changes occurring in the peripheral blood with the changes observed in the marrow. A significant decrease in the erythroid elements of the bone marrow begins at approximately 10 hr after irradiation and continues at a low level until approximately 150 hr. During this entire period of depression of the erythroid cells in the marrow, no appreciable reduction in the peripheral red blood cells is observed. It is only during the interval of most active marrow regeneration that the numbers of red blood cells in the peripheral blood are markedly reduced.

The appearance of nucleated red cells in the blood at a time when the mature cells are disappearing most rapidly suggests a stimulation of the bone marrow as a result of peripheral anemia. As the marrow becomes fully regenerated, there is some indication of hyperplasia, but this is transitory and regresses as soon as the red blood cells have attained their normal numbers.

Clarkson, Mayneord, and Parsons[18] have demonstrated that total-body irradiation is much more effective in reducing red cell count and hemoglobin than irradiation of localized parts of the body.

Reticulocytes have been frequently observed during the recovery period after radiation anemia.[5] The appearance of erythroblasts, on the other hand, has been reported in the circulating blood of animals but not in man during the period when the bone marrow still appeared essentially normal following large doses of irradiation.[7] In our experimental animals irradiated with 550 r, erythroblasts appeared in the peripheral blood in large numbers at about the time of the beginning of the bone-marrow regeneration and gave the first indication that the marked depression of the red blood cells was at an end. Normoblasts, however, made their appearance in the peripheral blood in significant numbers approximately 5 days after the appearance of the first erythroblasts. As would be expected, the nucleated red blood cells and the reticulocytes disappeared from the peripheral blood in direct proportion to the quantitative return of the normal red blood cells.

The deleterious effect of irradiation on the blood-forming organs is apparently the chief cause of the changes in the peripheral-blood picture. The best criterion of the functional activity of blood-forming organs is the maintenance of the normal blood, but any accurate appraisal of the state of the hematopoietic system requires parallel histological evaluation of the bone marrow.

The majority of investigators report a greater or lesser reduction in the parenchymatous cells of the bone marrow as a result of direct radiation. It is impossible, however, to form any definite impression from the literature as to whether the erythroblasts or the myeloid cells are affected first and to what extent. Many investigators hold that the cells of the myeloid series are more sensitive to irradiation than those of the erythroid series. Bauer[19] and Denstad[12] are the main proponents of a greater irradiation effect on the erythroid than on the myeloid cells.

The results of our experiments on rats are in agreement with the findings of Bauer and Denstad; the depression in the erythroid cells occurs somewhat earlier than in the myeloid cells, and the onset of regeneration of the erythroid cells precedes that of the myeloid cells. Heineke[1] reported that regeneration began in the bone marrow 12 to 14 days after irradiation, with the appearance of a large number of mitoses in this tissue. After 4 weeks the marrow was normal. Our investigations confirm the observations of Heineke.[1] Regeneration in the bone marrow in our animals began on the fifteenth day after irradiation and was almost completed on the forty-first day.

Wigoder,[20] Hu and Frazier,[21] Regaud and Blanc,[22–25] Schinz and Slotopolsky,[26] and Hertwig[6] have investigated the effects of irradiation on the testes in a wide variety of animals. There is considerable diversity of opinion as to the sequence of cellular degeneration in irradiated testes. One group of investigators[6,22–25] maintains that the spermatogonia are the cells which show the first signs of injury, followed by the spermatocytes and spermatids. Other investigators[20,21] have concluded that the spermatogonia are the last of the germinal cells to be affected and believe that the spermatocytes and spermatids are the most sensitive germinal cells. All agree that the Sertoli cells are little or not at all affected by irradiation.

In our animals irradiated with 550 r the spermatogonia in the early prophase stages of mitoses appear to be the most sensitive cells and show signs of irradiation injury as early as 4 to 6 hr after treatment. Of interest in this connection are the observations of Hertwig[6] on male mice irradiated with 800 r. She found that spermatogonia with the dusty type of nuclei were present in small numbers throughout the postirradiation period and believes that they are the stem cells for future regeneration. Spermatogonia with the crusty type of nuclei had already disappeared in her animals at 24 hr and did not reappear until 11 to 13 days after irradiation. In our animals the crusty type of spermatogonia completely disappeared at about 4 days and began to reappear at approximately 20 days.

The appearance of large numbers of degenerating cells in the proximal epididymides between the sixth and tenth postirradiation days and the presence of pycnotic cells and giant cells in the seminiferous tubules emphasize the fact that the eventual cellular depopulation is not entirely due to the destruction of the spermatogonia but that certain of the other spermatogenic cells must also suffer direct irradiation injury.[26]

Snell[27] irradiated mice with doses of 500 to 800 r and showed that the initial fertile period after irradiation lasted for approximately 2 weeks. Thereafter, the males were sterile for some weeks, and sperm were absent from the excurrent ducts. The regeneration of germinal cells after irradiation injury is very slow. Regeneration begins in the mouse testes at approximately 90 days[27] and in the rat testes at approximately 100 days.[28] In our animals sacrificed at 42 days after irradiation, although no active regeneration was occurring, mitotic division of the spermatogonia was occurring with some frequency.

A definite reduction in sperm motility did not begin in our animals until approximately 12 days after irradiation. Thereafter, a gradually increasing number of animals in which spermatozoan motility had decreased was observed. It was only after the thirty-fifth postirradiation day, however, that the sperm of all males examined were nonmotile. The loss of motility in the animals examined at this time is probably an indication of irradiation injury. Young[29] has shown that, in the normal guinea pig, sperm isolated in the epididymides retained their fertilizing capacity for approximately 35 days. He also demonstrated that the passage of sperm from the head to the tail of the epididymis is accomplished in 14 to 18 days.[30] With this information in mind, it may be assumed that, 20 days after irradiation, at least certain of the motile spermatozoa in the epididymides had completed maturation in the testes and epididymides in spite of irradiation. From the observations of Snell, it would be expected that those spermatozoa, although still motile after 12 to 14 days, would not have been able to effect fertilization.

The histopathological changes in the gastrointestinal tract after intensive irradiation have long been known in both experimental animals and man.[31] With heavy irradiation, not all parts of the intestinal tract show the same amount of damage. The stomach and colon show much less injury than the small intestine, and the proximal portions of the small intestine are ordinarily much more extensively injured than the distal parts. In the fundus of the stomach the primary reaction to irradiation is the cessation of mitoses and some pycnosis of the chief cells. In the small bowel, on the other hand, injury may be so extensive as to result in the degeneration of a large number of the basal glandular cells and subsequent thrombosis of the vessels, which may proceed to extensive necrosis and ulceration.[2]

5. SUMMARY AND CONCLUSIONS

This experiment was undertaken in an attempt to study the sequence of events during injury and repair to the body tissues of rats that had been acutely exposed to X rays (see Table 2.1).

The lymphatic tissues of the body suffered the most prompt and extensive injury, but it was also the tissue in which regeneration occurred most rapidly.

1. In the lymph nodes the effects of irradiation injury were clearly seen at 1 hr after irradiation. At 6 hr after irradiation, cytolysis of the cells in the follicles had reached its maximum, and the cellular debris was rapidly being removed by macrophages. At 24 hr after irradiation, regeneration and repair of the lymph nodes were actively progressing, and, by 17 days after irradiation, regeneration was complete.

Table 2.1 — Summary

Tissue	Time after irradiation at which injury was first observed	Time after irradiation at which injury reached its maximum	Time after irradiation at which regeneration began	Time after irradiation at which regeneration was completed
Spleen	30 min – 1 hr	20 – 30 hr	30 – 35 hr	40 days
Lymph nodes	30 min – 1 hr	6 hr	24 hr	17 days
Bone marrow	15 min	2.5 – 5 hr	12 days	40 days
Gastrointestinal tract	1 hr	4 – 6 hr	12 hr	30 hr
Testes	4 – 6 hr	30 – 35 days	42 days	
Blood and bone-marrow cells:				
Red blood cells	100 hr	15 days	15 days	32 days
Hemoglobin	100 hr	16 days	16 days	32 days
Lymphocytes	15 min	15 hr	11 days	41 days
Neutrophils	4.5 hr	4.5 days	4.5 days	20 days
Myeloid series	45 hr	90 hr	12.5 days	41 days
Erythroid series	6 hr	5.5 days	5.5 days	8 days
Megakaryocytes	1 – 3 hr	4 – 13 days	14 days	*
Platelets	70 hr	4 – 12 days	12.5 days	41 days

*Had not returned to normal at 41 days.

2. The changes in the spleen after irradiation followed closely those observed in the lymph nodes except that the whole process was somewhat more prolonged. Regeneration was first observed between 30 and 35 hr after irradiation and was not completed until 40 days after irradiation.

3. Cellular destruction in the bone marrow reached its maximum between 2½ and 5 hr after irradiation. Most of the accumulated cellular debris was removed by the thirtieth postexposure hour. By the eighth day, hypoplasia was very severe, but at no time was aplasia observed. Active regeneration began at approximately 12 days after irradiation and was completed at 40 days.

4. A decline in the number of peripheral red blood cells began at 100 hr after irradiation and continued for 15 days. Thereafter, regeneration was gradual and reached approximately normal levels at 35 days. During the period of the greatest depression of the red cells in the peripheral blood, nucleated red blood cells and reticulocytes were present in large numbers. The fall in the blood hemoglobin followed closely the decline of the red blood cells.

5. A reduction in the number of lymphocytes in the peripheral blood was already observed in animals killed 15 min after irradiation. The reduction in the number of lymphocytes progressed at a rapid rate so that by 15 hr after irradiation the blood was almost devoid of these cells. The marked depression of the blood lymphocytes continued for approximately 10 days, after which recovery began. Recovery was slow, and normal numbers of lymphocytes were not observed until 41 days after irradiation.

6. A transient leucocytosis was observed during the first few hours after irradiation and was caused by an increase in the numbers of circulating neutrophils. Neutropenia began at 4½ hr after irradiation and continued for 4½ days. The return of the neutrophils to the peripheral blood was gradual but occurred at a more rapid rate than the return of the lymphocytes.

7. A reduction in the number of erythroid cells in the bone marrow occurred at a considerably earlier period than in the myeloid cells. Regeneration of the erythroid cells also began somewhat earlier than the myeloid cells. In general, the cellular changes that occurred in the irradiated bone marrow were reflected in the changes observed in the cells of the peripheral blood.

8. The decrease in the number of megakaryocytes in the bone marrow followed closely the general reduction in marrow cellularity. The greatest reaction occurred between the fourth and twelfth days, after which regeneration began and was completed at approximately the forty-first day.

9. The reduction in the number of platelets in the peripheral blood is associated with the decline in the number of megakaryocytes in the marrow.

10. The spermatogonia in the early prophase stages of mitoses first showed injury between 4 to 6 hr after irradiation. Spermatogonia with the crusty type of nuclei disappeared from the seminiferous tubules 4 days after irradiation and reappeared at approximately 20 days. Active mitosis was observed in the spermatogonia 42 days after irradiation.

11. In the ovary the granulosa cells of the intermediate and small follicles are the cells most sensitive to acute irradiation.

12. In the digestive tract the small intestine suffers the greatest injury following acute irradiation. Although there was marked cytolysis of the basal cells of the villi, neither thrombosis nor ulceration was observed in the tissue sections. Repair of the injured bowel occurred rapidly and was completed 30 hr after irradiation.

13. No significant injury was observed in the kidneys, lung, liver, or bone as the result of irradiation.

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Chapter 3*

EFFECTS OF ACUTE EXPOSURE TO X RADIATION ON THE PERIPHERAL BLOOD OF EXPERIMENTAL ANIMALS

By M. Ingram and W. B. Mason

1. INTRODUCTION

At the time this study was undertaken an unprecedented number of persons were potentially exposed to radiation during their daily work. Rigorous evaluation of the safe doses of the different radiations and the development of practical, reliable methods for monitoring radiation exposure were consequently of utmost importance in protecting the health of exposed individuals or those likely to suffer exposure. The widely recognized effectiveness of radiation in producing changes in the hematopoietic tissues and a reduction in circulating formed elements of the blood directed attention toward hematology as a possible means of detecting overexposure to radiation, and it was largely for this reason that these studies and those reported in Chap. 11 were undertaken.

From the beginning, these experiments, which were planned and carried out under the direction of F. A. Bryan and G. M. Suter, were designed to give information on the problem of the hematological effects of acute exposure to whole-body X radiation. The purposes of the experiments were, first, to determine the changes that occur in the hemograms of animals exposed to various amounts of radiation and, second, to decide whether or not it would be possible to translate the experimental findings into a practical monitoring system that would utilize changes in the worker as an indication of his radiation exposure.

In addition to providing the desired information relative to the sensitivity of the hematopoietic tissues to irradiation, the experimental results clearly indicate the danger of relying solely on routine hematology as a method of monitoring.

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*Based on Report UR-122, July 17, 1950.

2. METHODS

Rats, dogs, and monkeys were used as experimental animals. Animal selection, care, and feeding were essentially the same, as reported in detail in Chap. 9.

X rays were delivered from a Picker industrial machine operated at 250 kvp (kilovolts peak) and 15 ma. A 14.2-mm aluminum parabolic filter, plus a 0.53-mm copper filter, was used in all instances, giving a beam having a half-value layer (HVL) of 2.15 mm copper. Radiation was delivered at the rate of approximately 7 r/min. The dose measured in the rat experiments was the dose delivered in air, as measured by a Victoreen ionization chamber at the level of the floor of the cage in which the animals were irradiated. The target-skin distance was 42 in. In the case of dogs the dose delivered in air was measured at a distance corresponding to that from tube target to the center of the animal; the target–dog-center distance was 40 in.

A complete blood count included enumeration of the erythrocytes, leucocytes, reticulocytes, and platelets; hemoglobin determination; differential leucocyte count; and an appraisal of poikilocytosis, anisocytosis, basophilia, and stippling. The counts were all made according to standard procedures. Hemoglobin determinations were made on a Klett-Summerson colorimeter. Differential leucocyte counts were based on the classification of 100 cells on a Wright's stained blood smear. Reticulocyte counts were made on Wright's stained cover-slip smears made by using one plain cover slip and one cover slip on which a drop of brilliant cresyl blue had been allowed to dry. The number of reticulocytes found in 1000 cells was determined, and the incidence was recorded in percentage.

2.1 Studies of Rats. The studies of rats were more extensive than those involving either dogs or monkeys. Rats of the Wistar strain were used exclusively. Only those animals weighing about 140 g (6 to 8 weeks of age) and having preirradiation leucocyte counts in the range of 10,000 to 20,000 cells/cu mm were selected for experimental use.

Rats were bled by cutting approximately $\frac{1}{8}$ in. off the end of the tail. All smears and dilutions for counts were prepared from freely flowing blood. Each animal had one or two complete blood counts before the beginning of the experiment.

The experiments were originally designed so that the largest dosage would exceed the LD_{100}. Graded dosages ranged from this maximum down to a dose that was not expected to have any apparent effect on the counts of circulating cells. These limits were initially set at 1500 and 25 r. At the completion of the original experiments, however, the lowest dosage was found to have produced significant changes within the first 48 hr following irradiation, and the studies were then supplemented by a series of observations based on dosages ranging from 25 to 5 r.

In the initial experiment there were 13 groups of 18 animals each. The various groups received, respectively, 1500, 1250, 1000, 800, 700, 600, 500, 400, 300, 200, 100, 50, and 25 r of whole-body radiation. Postirradiation blood counts were made approximately every 2 days for the first 12 days and then weekly for 2 weeks, so that the duration of the entire experiment was roughly 1 month. All blood samples were obtained between 8:00 a.m. and noon. In the supplementary experiment there were five groups of animals composed of 16 to 40 animals each. One group of 40 animals served as a control group, and the other groups received, respectively, 25.0, 17.5, 10.0, and 5.0 r of whole-body X radiation. Each animal had 13 postirradiation blood counts spaced at intervals of 4 hr.

Hematological data were also collected on rats studied for pathological changes following a single dose of whole-body X radiation approximating LD_{50} (550 r). The protocol for this experiment was complicated by the fact that rats cannot be bled frequently without losing a large fraction of their blood volume, thereby introducing effects due to hemorrhage. In order to minimize this effect the blood counts of the individual groups were staggered in such a manner that, although most animals were bled not more than seven times during the entire experiment, the over-all postirradiation picture was based on counts made every 15 min for the first hour, every ½ hr from 1 to 8 hr, every hour through 43 hr, every 6 hr up to 122 hr (5 days), and daily from 5 to 42 days. Two rats in each group were sacrificed at a time corresponding to the last count for that group. Pathological studies are reported in Chap. 2.

2.2 Studies of Dogs. The hematological effects of acute exposure to whole-body X radiation were studied in 25 dogs that received 300 r, a dose approximating the LD_{50}. These animals were control groups in an investigation carried out at the Rochester Project by P. E. Rekers. Blood was obtained from the jugular vein by venepuncture. Dilutions for platelet counts and cover-slip smears for Wright's staining and reticulocyte counts were made directly from blood flowing from the needle. All other counts were made on oxalated blood. Each animal had at least two complete blood counts before irradiation. Complete hemograms were determined every 2 to 3 days for 60 days following exposure. Bone-marrow samples were obtained from a comparable group of animals by consecutive rib biopsies performed 2 and 4 days after irradiation and thereafter at approximately weekly intervals for the remainder of the 60 days. Marrow sections were also obtained from any animal that died during the postirradiation experimental period.

2.3 Studies of Monkeys. Monkeys were exposed to single doses of X radiation in amounts ranging from 2000 to 300 r. There were eight groups of four animals each of which received, respectively, 2000, 1500, 1325, 850, 750, 450, 375, and 300 r of whole-body radiation. Blood was obtained from the femoral vein by venepuncture. Cover-slip smears and dilutions for platelet counts were prepared from blood flowing freely from the needle in the vein. All other counts were made on oxalated blood. Every animal had one complete blood count 3 days before exposure. Postirradiation hemograms were determined on the day of exposure and thereafter daily for 2 weeks, then twice weekly for the following 2 weeks, and once during the fifth week, after which the experiment was concluded.

3. FACTORS INFLUENCING INTERPRETATION OF RESULTS

Many difficulties and opportunities for the introduction of extraneous factors that may influence the experimental findings are encountered in carrying out long-range studies of the magnitude of those presented here and in Chap. 11. Certain of the problems in this chapter have proved to be of importance in the interpretation of experimental results and for this reason are discussed briefly.

The monkeys were disappointing throughout. In addition to being difficult to handle, they proved to have high susceptibility to respiratory infections, particularly tuberculosis. Mortality was high, and the general health of the entire group was consistently poor during the period of observation. At the completion of the experiments, when the data were subjected to analysis and statistical testing, it was readily apparent that the hematological data could not be interpreted as illustrating significant changes due to irradiation. Consequently further interpretation was not attempted, and the experiments are not discussed in detail. (Studies on changes in finger ridge detail in the monkey are reported in Chap. 6.)

The necessity for frequent bleeding in rat experiments of short duration introduces the possibility of changes in the hemograms due to blood loss. In order to minimize this possibility the experiment in which the rats received a single dose of 550 r was arranged in such a manner that counts made at various intervals on the several groups would dovetail to produce the over-all postirradiation picture. This resulted in a complicated protocol and introduced a significant variable to be considered in the analysis of results.

When the statistical analyses were completed at the end of the supplementary experiment, a pronounced diurnal variation was noted in the leucocyte count of the control rats (Fig. 3.1). This is of special importance in the interpretation of results in the supplementary experiments in which the observations were limited to the first 52 hr following irradiation.

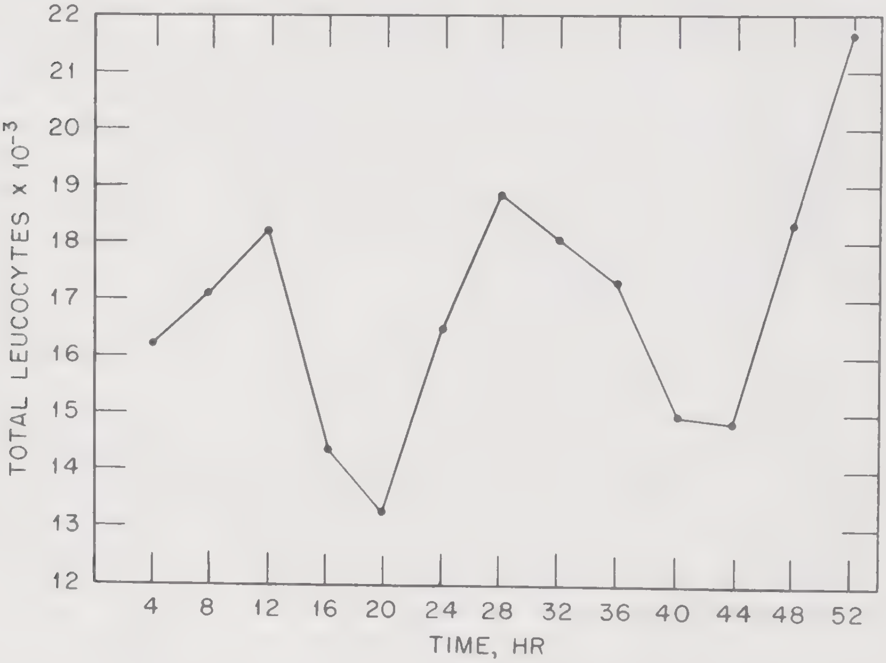

Fig. 3.1—Diurnal variation in the total leucocyte count of control rats.

During severe leucopenia, when the total leucocyte count was extremely low, it was often difficult to find enough leucocytes in a smear to make an adequate differential count. In instances in which fewer than 50 cells were classified, the reported differential count must be considered with appropriate reservation. This restriction applies to a large share of the differentials determined during severely leucopenic phases in the rat experiment.

Ideally, interpretation of the results would have been accomplished, first, by comparing preirradiation and postirradiation values of a given variable and, second, by comparing the trend of a given variable with trends of the same variable in a control group. This was possible, however, only in the supplementary experiment in which rats received single doses of whole-body radiation in amounts ranging from 25 to 5 r. In other experiments, interpretation was accomplished by using mean preirradiation counts as reference points with which to compare postexposure mean counts. This method is not entirely satisfactory, particularly when trends are not well defined and are "significant" by statistical methods only. Unfortunately the necessity for statistical evaluation of the data was apparent only after the experiments were almost completed. If this had been anticipated, the usual one or two preexposure counts could have been extended to a large series for each animal, which would have permitted a much more quantitative interpretation of the data.

4. RESULTS

Much of the interpretation of results is based on statistical analysis. The methods used are not described in detail, but a brief description of the statistical approach to the problem may be helpful in understanding the interpretations. The analysis-of-variance technique has been used throughout. In the data being tested, each point on its graph represents a mean value for a group of counts. The analysis-of-variance technique consists in determining the range of variation around each point on the graph and comparing that variation with the change between successive points. The variability around single points thus provides an indication of the significance of variability between points. Any trend that is statistically significant for a given graph is then further compared with the data from the control group. If statistically significant changes of equal magnitude are present in the control group, the trend in the experimental group cannot be interpreted as representing a result of treatment.

An important consideration in the interpretation of results relates to the condition that many of the experimental groups underwent marked reduction in size owing to death of the animals during the course of the experiments. This change was especially prominent in those groups of rats which received radiation in amounts greater than the LD_{50}. The mortality data pertaining to these animals are summarized in Fig. 3.2. It will be noted that, of the 108 rats divided equally among the six groups that received X radiation in amounts greater than the LD_{50}, only 4 rats survived the 25-day postirradiation period during which hematological observations were made.

The group of rats that received radiation in an amount approximating the LD_{50} (550 r) also showed a high mortality. The nature of the protocol makes it impossible to determine the over-all mortality, but in the case of the 16 animals used to supply information relative to the interval extending from the seventh to forty-second postirradiation days, deaths began on the eighth day, and half the animals were dead by the eleventh day. By the eighteenth day, only three animals remained, and all these survived for the duration of the experiment.

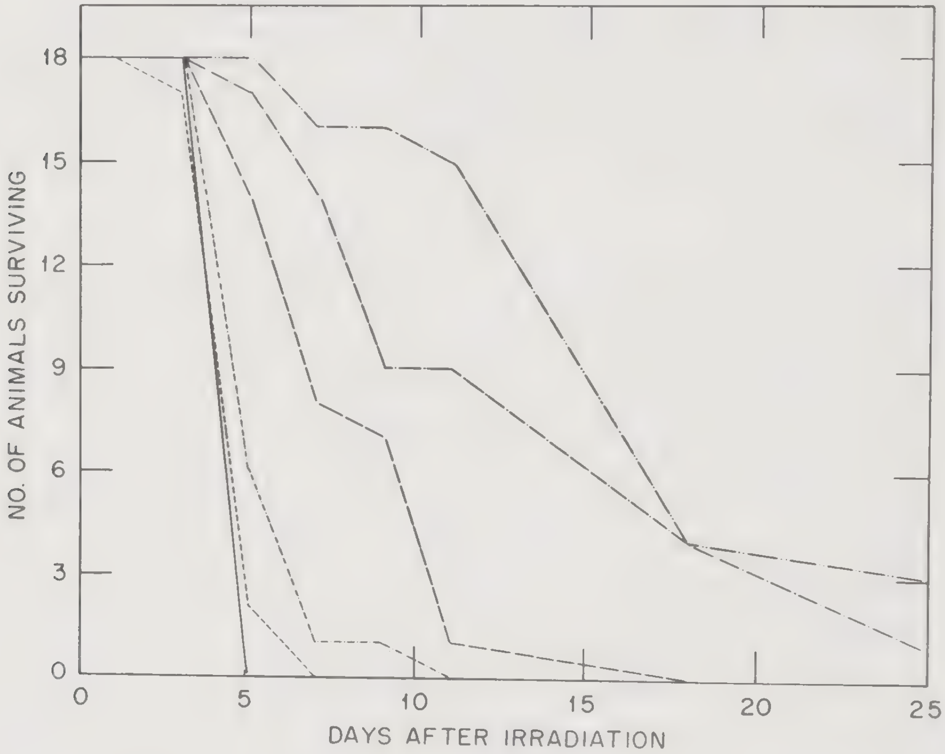

Fig. 3.2—Mortality of rats after a single exposure to X radiation in doses greater than LD$_{50}$. ——, 1500 r. -----, 1250 r. ·–·–, 1000 r. ---, 800 r. .—., 700 r. –··–, 600 r.

By comparison there were only five deaths among the 126 rats that composed the groups receiving radiation in amounts less than the LD$_{50}$. All except one of these deaths occurred during the last week of the experiment, during which time one animal each died in the 500-, 400-, 300-, and 200-r groups. The other death occurred in the 500-r group between the twelfth and eighteenth postirradiation days.

From this description of the mortality in the various groups, it is clear that different phases of the hematological response to acute exposure to X radiation are not demonstrated with equal certainty. For example, the early downward trends in the peripheral-blood elements of rats that received amounts of radiation approximating or exceeding the LD_{50} are more reliable than trends developing during the recovery phase, when only a few animals were still alive. By contrast, all phases of the response to doses of radiation less than the LD_{50} are equally significant. Furthermore, in the higher-dosage groups there is the question of whether or not the hematological changes are essentially the same in surviving and dying animals. This question has been considered statistically in the 600-r rat group and the 300-r dog group. In these instances there are no consistent statistically significant differences between the hematological changes observed in surviving and dying animals, although minor differences are to be noted. The mortality in other groups is such that it is not feasible to compare changes in surviving and dying animals.

Interpretation of observed changes is often difficult. For example, the temporarily increased erythrocyte counts in rats exposed to doses greater than 500 r may reflect hemoconcentration, dehydration, or other alterations in fluid balance and do not necessarily imply an absolute increase in the number of erythrocytes in the peripheral blood. For convenience in description, increases in erythrocyte counts are referred to as erythrocytoses, without any attempt to specify the underlying mechanism.

The following descriptions of the changes that occurred in the hemograms of rats and dogs following single exposures to various amounts of whole-body X radiation are based on the mean counts in all animals surviving at the time under consideration.

4.1 Lymphocyte Trends in Rats Irradiated with X Rays. Depression of the lymphocyte counts preceded the depression of counts of other leucocytes of the peripheral blood and was of greater magnitude.

(a) Doses Greater than LD_{50} (1500 to 600 r). Lymphocyte counts decreased abruptly within 24 hr in all animals treated with 1500 to 600 r. The 1500-, 1250-, 1000-, and 800-r animals all died during the period of profound leucopenia, with absolute lymphocyte counts in the range of 100 to 200/cu mm (approximately 1 to 3 per cent of the mean preexposure counts). A definite increase in the counts of surviving animals was first noted on about the eighteenth day. The counts continued to increase thereafter, although they had not reached normal levels in either of the surviving groups (700 and 600 r) when the experiment ended on the twenty-fifth postexposure day.

(b) Dose Approximating LD_{50} (550 r). Statistically significant depression of lymphocytes was demonstrated within 15 min after irradiation in rats exposed to the midlethal dose (550 r) of X rays. The absolute lymphocyte count decreased progressively thereafter and reached minimal levels at 11 to 15 hr. These minimal levels, ranging from approximately 1 to 3 per cent of the preexposure counts, were maintained until approximately 10 days after exposure, when the absolute lymphocyte count in the peripheral blood began to increase steadily. On the twenty-fifth postexposure day the lymphocyte counts were approximately 60 per cent of the preexposure counts. The trend continued upward, and, when the experiment ended on the forty-second day, the counts approximated the preexposure level.

(c) Doses Less than LD_{50} (500 to 25 r). Lymphocyte depression was maximal approximately 24 hr after exposure except in the 500- and 400-r groups, in which a slight further decrease was observed during the following 2 days. Depressions were progressively less marked with decreasing dosages from 500 to 25 r. In all groups a gradual return toward normal began after the third day (seventy-fifth hour) following irradiation, and the rate of increase differed very little from group to group. A stratification of counts in which depressions were greater in the higher-dosage groups persisted throughout the period of recovery and was still apparent at the completion of the experiment on the twenty-fifth postirradiation day. At that time none of the counts had returned to their preirradiation levels. The maximum depression of counts and the extent of recovery by the twenty-fifth day are given in Table 3.1.

Table 3.1 — Lymphocyte Depression and Extent of Recovery

Dosage group, r	Minimal mean lymphocyte count*	Mean lymphocyte count* on 25th day
500	1	40
400	3	59
300	6	53
200	7	54
100	27	75
50	39	83
25	73	85

* Percentage of the preexposure mean count.

(d) Supplementary Experiment (25 to 5 r). Lymphocytes were significantly depressed within 15 min after treatment in all rats exposed to doses ranging from 25 to 5 r. The depressions were superimposed on the diurnal variation in the leucocyte count and were not readily apparent. By inspection, however, the depressions appeared to be proportional to the dosage. The nature of the data is such that it is not possible to determine whether or not the period of depressed counts extended beyond the period of observation, i.e., 52 hr following exposure.

(e) Lowest Dose Affecting Lymphocytes. Although the minimal effective dose was not determined in these experiments, the smallest dosage investigated, 5 r, was sufficient to produce a highly statistically significant depression of the lymphocytes. The changes observed were superimposed on the marked diurnal variation in the leucocyte counts and were demonstrable only when the data were treated statistically. As already noted, the depression was effected within 15 min after irradiation.

4.2 Neutrophil Trends in Rats Irradiated with X Rays. Neutrophils decreased significantly in all rats exposed to single doses of X rays in amounts ranging from 1500 to 50 r. In all cases depression of the neutrophil counts occurred later than the corresponding depression of the lymphocyte counts.

(a) Doses Greater than LD_{50} (1500 to 600 r). The neutrophils decreased precipitously in all groups of rats treated with doses ranging from 1500 to 600 r. In general, the absolute neutrophil counts were minimal by the third to fourth days and varied little until approximately the eighteenth to nineteenth days, at which time they showed a definite increase in all survivors. All animals exposed to 1500 to 800 r died during the period of severe leucopenia. The absolute neutrophil counts of surviving animals approached normal levels by the end of the experiment (25 days).

(b) Dose Approximating LD_{50} (550 r). The first observable change induced in the absolute neutrophil counts by exposure to the midlethal dose of radiation was a transient increase that began within 2 hr following irradiation. Counts returned to normal at 14 hr and, after a brief stable period in that range, began an irregular downward course approximately 21 hr after treatment. Minimal absolute neutrophil counts prevailed from approximately the third to tenth days, when counts were roughly 5 to 7 per cent of the normal preirradiation levels. A definite trend upward began approximately 12 days after exposure, and values reached normal at approximately 16 days. The counts continued to increase and attained levels slightly above the preexposure level at 18 to 19 days after exposure. Thereafter they fluctuated above and below normal levels.

(c) Doses Less than LD_{50} (500 to 50 r). Neutrophil counts were depressed in all groups irradiated with 500 to 50 r. The magnitude of the depression varied from group to group within the first 24 hr, and during this interval there was no relation between the magnitude of the depression and the dose administered. The depressions became more marked during the second and third days and showed a correlation with increasing dosages. Thereafter the counts tended to return toward normal in a more or less erratic manner, and all approached or exceeded the preexposure values by the twenty-fifth day. Table 3.2 summarizes the maximum depression of the neutrophil counts and the extent of recovery on the twenty-fifth postexposure day in the 700- to 50-r dosage groups.

(d) Supplementary Experiment (25 to 5 r). Neutrophil counts were not significantly depressed during the 52-hr period following exposure to doses ranging from 25 to 5 r.

Table 3.2 — Neutrophil Depression and Extent of Recovery

Dosage group, r	Minimal mean neutrophil count*	Mean neutrophil count* on 25th day
700	1	108
600	3	89
500	7	93
400	15	87
300	34	120
200	33	99
100	70	100
50	75	103

* Percentage of preexposure mean count.

(e) Lowest Dose Affecting Neutrophils. The lowest dose affecting the neutrophils in this experiment was 50 r, which produced a very transient depression during the 3 days following irradiation. Mean counts during that interval were 75 to 77 per cent of the mean preexposure counts.

4.3 Total Leucocyte Trends in Rats Irradiated with X Rays. The total leucocyte trends paralleled the variation in the lymphocyte counts in direction and magnitude in all treatment groups. This might be expected in view of the fact that in the rat the lymphocytes normally comprise 70 to 80 per cent of the total white blood cell count. Any differences noted between the total leucocyte and lymphocyte trends were so slight that their significance is questionable. The similarity of the two trends persisted even in the supplementary experiment in which the doses ranged from 25 to 5 r. In that study both the lymphocytes and the total leucocytes showed significant depressions. The only difference observed in the responses of the lymphocytes and total leucocytes was that the lowest dose effecting a depression in the case of the former was 5 r, and in the latter, 10 r. The slightly higher dose required to depress the total leucocytes probably represents the influence of the neutrophils that were not affected by the doses used in this experiment.

4.4 Platelet Trends in Rats Irradiated with X Rays. In general, the platelet counts were reduced following exposure to X rays. Thrombopenia characteristically began later than leucopenia.

(a) Doses Greater than LD_{50} (1500 to 700 r). A pronounced thrombopenia occurred in rats treated with 1250 to 700 r, and in all groups the depression was preceded by a temporary rise during the third postexposure day. Only in the 1500- to 800-r treatment groups, however, did the increased counts exceed the normal levels. During the first 24 hr after treatment the counts in all except the 1500-r group decreased slightly. There was no definite relation between the dose and the magnitude of either the initial 24-hr depression or the third-day rise in counts. All animals in the 1500-r group died during the period of increased platelet counts; consequently no thrombopenia was demonstrable. Counts in the 1250- to 700-r groups, however, decreased abruptly after the transient rise and, except for the 1250-r group in which all rats died during the period of decreasing counts, reached minimal levels, ranging from 6 to 15 per cent of their preexposure counts, approximately 8 days after exposure. Only in the 700-r group did any of the animals survive the period of depressed counts. In this group, platelet counts began to increase at approximately 18 to 19 days. They had not returned to normal, however, by the twenty-fifth postirradiation day, when the experiment ended. Table 3.3 indicates the count on the third day, the extent of maximum depression, and recovery at 25 days.

(b) Doses Approximating LD_{50} (600, 550, 500 r). The trends of the platelet counts following irradiation in the 600-, 550-, and 500-r groups are closely similar. In all groups there was very slight depression of the count until approximately the fourth day, when the count began to decrease steadily. The initial increase observed in the 1500- to 800-r treatment groups was not demonstrated.

Marked depression of the platelet counts was present by the end of the first week and continued into the third postexposure week. Minimal counts ranged from approximately 11 to 16 per cent of the mean preexposure counts in the 600- and 550-r groups and were approximately 25 per cent of the preexposure counts in the 500-r group. During the third week the platelets began to increase in all groups, and on the twenty-fifth day, when the experiment ended, the counts were approximately 50, 62, and 80 per cent of the preexposure counts in the 600-, 550-, and 500-r groups, respectively.*

(c) Doses Less than LD_{50} (400 to 25 r). Depression of platelets in rats treated with 400 to 100 r appears to be roughly proportional to the dose. In groups treated with 50 to 25 r, platelets were increased slightly over normal values. In view of the many sources of error in platelet counting, however, the variations in the 50- and 25-r groups are probably not significant. The total period of depression of the platelet counts in the lower-dosage groups was essentially the same as in the LD_{50} groups. Minimal counts prevailed from the ninth to twelfth days. Counts of all groups approached or reached normal by the twenty-fifth day.

Table 3.3 — Trends of Platelet Count

Dosage group, r	Mean platelet count* on 3rd postexposure day	Minimal mean platelet count*	Mean platelet count* on 25th day
1500	152	†	†
1250	112	†	†
1000	107	†	†
800	131	†	†
700	87	6	26
600	72	15	50
500	93	25	84
400	80	36	95
300	94	32	88
200	100	40	95
100	96	60	81
50	134	‡	119
25	127	‡	95

* Percentage of preexposure mean count.
† Died before period of maximum depression of platelets.
‡ No decrease.

The magnitude of the greatest depression for all groups and the extent of recovery at the end of the experiment are summarized in Table 3.3.

(d) Lowest Dose Affecting Platelets. No significance can be attached to the minor variations in the platelet counts of rats irradiated with 50 to 25 r. In these experiments 100 r was the lowest dose producing a significant decrease in platelets. The resultant trend is described with the effects of doses less than LD_{50}.

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*In the 550-r group the count was taken on the twenty-eighth day.

4.5 Erythrocyte and Hemoglobin Trends in Rats Irradiated with X Rays. Erythrocyte counts of rats were depressed only in groups treated with doses greater than 300 r. In all cases the hemoglobin changes were roughly parallel to the trends of the erythrocyte counts. The error inherent in the hemoglobin determination invalidates any attempt to attach significance to the minor differences observed between the two trends. For this reason the trends of only the red blood cell count are discussed.

(a) Doses Greater than LD_{50} (1500 to 600 r). In general, erythrocyte counts decreased markedly following exposure to 1500 to 600 r of X radiation. The anemic phase, however, was preceded by a brief period of increased red blood cell counts. Maximal counts were observed approximately 3 days after exposure, at which time the increases were more marked in the higher-dosage groups. All animals in the 1500- and 1250-r groups died during the period of erythrocytosis and consequently did not develop anemia. In all other groups, however, following the temporary erythrocytosis, counts diminished progressively toward minimum levels that were reached between the eleventh and seventeenth postexposure days. Red blood cell counts subsequently increased in surviving groups, although they had not reached normal levels by the twenty-fifth day, when the experiment ended. Counts of surviving animals (700- and 600-r groups) were roughly proportional to the dosage at the time of maximal depression of counts and at the end of the experiment. Table 3.4 indicates the extent of the preliminary erythrocytosis, the depression during the phase of decreasing counts (ninth day), minimal counts, and counts on the twenty-fifth day. The trends of the 500-, 400-, and 300-r groups are similar to those described above, and the data for these groups are included in Table 3.4.

(b) Dose Approximating LD_{50} (550 r). The red blood cells began to decrease approximately 5 days after exposure to 550 r. No definite period of erythrocytosis was apparent in these animals, and the nature of the data is such that no significance can be attached to the minor fluctuations, above and below the preirradiation level, which occurred during the first 5 days.

From the tenth to fifteenth days, counts were minimal and ranged from 23 to 29 per cent of the mean preexposure count. A slow but definite increase in counts was observed after the seventeenth day, and on the twenty-eighth day the mean red blood cell count had reached 73 per cent of the preexposure mean count.

(c) Doses Less than LD_{50} (500 to 25 r). Significant depression of the red blood cell counts occurred in rats treated with 500 to 300 r.

Depression of the counts was definite by the ninth postexposure day in these groups, and minimal counts were observed between the eleventh and nineteenth days. Thereafter the counts rose and, in the 300-r group, approached the preexposure level by the end of the experiment.

Table 3.4 — Trends of the Red Blood Cell Counts

Dosage group, r	Maximum mean RBC count*	Mean RBC count* at approximately 9 days	Minimal mean RBC count*	Mean RBC count* on 25th day
1500	127	†	†	†
1250	126	†	†	†
1000	115	40	‡	‡
800	126	43	‡	‡
700	105	56	27	59
600	101	54	35	69
500	103	83	54	71
400	103	80	70	89
300	100	85	82	97

* Percentage of preexposure mean count.

† Animals died during period of erythrocytosis.

‡ Animals died during period of decreasing counts and before minimal levels were reached.

Table 3.5 — Trends of the Red Blood Cell Counts

Dosage group, r	Mean RBC count* on 11th day	Mean RBC count* on 25th day
500	67	71
400	70	89
300	82	97
200	93	96
100	98	96
50	108	103
25	110	105

* Percentage of the preexposure mean count.

In the 500- and 400-r groups the counts were still depressed on the twenty-fifth day (Table 3.5).

Only the 500-r-group rats developed an initial erythrocytosis, and it was minimal. The 200- and 100-r treatment groups showed slight sustained decreases in the red blood cell counts later in the experimental period, and the 50- and 25-r groups showed slightly increased counts. No significance can be attached to the minor changes in these four groups, however. After the ninth day there was a stratification of counts in which counts were higher in the lower-dosage groups. Table 3.5 gives the counts on the eleventh and twenty-fifth days.

(d) Lowest Dose Affecting Erythrocytes. Doses smaller than 300 r failed to produce a significant reduction in erythrocytes during the period of observation. The changes in the 300-r group are described with the effects of dosages less than LD_{50}.

4.6 Reticulocyte Trends in Rats Irradiated with X Rays. In general, reticulocytes resemble lymphocytes in regard to the promptness and magnitude of their depression following single exposures to whole-body radiation.

(a) Doses Greater than LD_{50} (1500 to 600 r). Reticulocytes decreased markedly within 24 hr in all rats irradiated with doses ranging from 1500 to 600 r. The depressions were not proportional to dosage. Maximum depression was observed in all groups on the third postexposure day, at which time the counts ranged from 0.4 to 2.0 per cent of preirradiation counts. Extremely low counts were maintained until the ninth to eleventh days after irradiation, at which time they began to increase very rapidly in survivors (700- and 600-r groups). At the end of the experiment the counts were still rising in these groups and were, respectively, 235 and 123 per cent of the preexposure mean counts. All animals in the higher-dosage groups died before the reticulocyte recovery trends began.

(b) Doses Approximating LD_{50} (550 r). As already noted, the reticulocyte trend resembled the lymphocyte trend in the promptness of the response and the magnitude of the maximum depression. In the LD_{50} experiment, however, in which counts were made at very short intervals during the first 5 days, it is possible to demonstrate a slight but definite difference between the two downward trends. Although both elements showed definite depressions within 15 min after exposure, the lymphocyte counts decreased steadily and reached minimal levels of 1 to 3 per cent of the preexposure counts at 11 to 15 hr after exposure. The reticulocytes, on the other hand, tended to stabilize after the initial drop and remained relatively constant until 15 hr after exposure, when they again began to decrease. They reached minimal levels on the third day. Reticulocyte counts thereafter ranged from approximately 1 to 4 per cent of preexposure counts until the eighth postexposure day, at which time reticulocytes began to appear in the blood in rapidly increasing numbers. By the twelfth day the counts exceeded the preirradiation level. Maximum counts, observed between the sixteenth and twenty-eighth postexposure days, varied from 500 to 700 per cent of the preexposure levels. Subsequently the counts tended to return toward normal, although on the forty-second postexposure day they were still 73 per cent above the original level. It may be noted that the reticulocyte and lymphocyte trends differed again in the recovery phase. The lymphocyte recovery began approximately 10 days after exposure and was characterized by a gradual increase with values just approaching normal levels on the forty-second postexposure day. The reticulocyte recovery, however, began approximately 8 days after exposure, and the counts increased very rapidly, reaching levels well above normal.

The magnitude of the reticulocyte increase in this group of animals was higher than that in the 600- and 500-r groups. The apparent discrepancy may, in part at least, be attributed to the difference in protocols. In the 550-r experiment, counts were made over a period of 42 days. In the 600- and 500-r groups the duration of the experiment was only 25 days, and reticulocytes were still rising slowly when the experiment ended. In the latter instances the peak of the reticulocyte trend may not have been reached during the period of observation. The erythropoietic stimulus provided by frequently repeated blood sampling in the 500-r group may also have contributed to the reticulocytosis in these animals.

(c) Doses Less than LD_{50} (500 to 25 r). The depression of the reticulocytes occurred within 24 hr after exposure and was roughly proportional to the dose in rats exposed to doses ranging from 500 to 25 r. Depressions were progressive from the first to approximately the third day and reached minimal levels 75 hr after irradiation in the 500- to 100-r groups. In the 50- and 25-r groups, depressions were small and difficult to evaluate. Recovery from depressed counts began on the ninth day in the 500-r group, on the seventh day in the 400-r group, and on approximately the fifth day in the 300- to 25-r groups. Reticulocyte counts in rats irradiated with 300 to 25 r were slightly erratic during the upward recovery trends, but all attained levels slightly above preexposure means and then approached normal levels. The increase above normal was greater in the 500- and 400-r groups, and in the former the counts were still rising at the end of the experiment.

Table 3.6 presents the extent of depression and recovery of reticulocyte counts of rats irradiated with doses ranging from 1500 to 25 r.

(d) Lowest Dose Affecting Reticulocytes. The 50-r treatment group showed a definite reticulocyte decrease after irradiation. Interpretation of data from the 25-r group is somewhat difficult owing to a second decrease in reticulocytes that occurred on the twenty-fifth day. In the 25-r group there appears, however, to have been a slight decrease (76 to 74 per cent of preexposure counts) during the first to

Table 3.6 — Reticulocyte Depression and Recovery

Dosage group, r	Minimal mean reticulocyte count*	Maximal reticulocyte count* during recovery phase
1500	1.5	†
1250	1.1	†
1000	1.5	†
800	0.6	†
700	0.9	235‡
600	0.4	123‡
500	5	284‡
400	6	283
300	9	134
200	18	166
100	50	107
50	74	112
25	74	103

* Percentage of the preexposure mean count.

† All animals in the group died while reticulocyte counts were depressed.

‡ Counts still rising at the end of the experiment.

third postirradiation days, which was subsequently followed by a rise to slightly above the normal level. Lower-dosage groups were not studied in regard to reticulocyte trends.

4.7 Trends of Cellular Elements of the Peripheral Blood of Dogs Irradiated with X Rays in Doses Approximating LD_{50}. In the case of dogs the information relative to the effects of acute exposure to X radiation was obtained from animals that received 300 r, a dose near the LD_{50}. The experiment probably does not represent the true LD_{50} effect since, of the 25 animals comprising the original experimental group, only 10 were living on the twenty-ninth day and only 9 on the thirty-second day. At the end of the experiment there were only four survivors.

(a) Lymphocytes. A precipitous decrease in lymphocyte counts began within 24 hr after exposure, and minimum counts of 22 per cent of the mean preexposure counts were attained by the second postexposure day. Lymphopenia persisted, with counts approximately 20 to 30 per cent of the preexposure means, until the eighteenth to twenty-first postexposure days, when the number of peripheral lymphocytes began to increase slowly and steadily. At the end of the experiment, 60 days after irradiation, the lymphocyte counts had still not reached normal levels in the surviving animals. On the fifty-third, fifty-seventh, and sixtieth days the counts were, respectively, 62, 83, and 50 per cent of the preexposure mean counts.

(b) Neutrophils. Neutrophil counts were depressed to 70 per cent of the preexposure mean count within 2 days after irradiation. The counts then fell abruptly and from the seventh to twenty-first days remained at a level of approximately 5 to 15 per cent of the preexposure means. Thereafter the neutrophils increased, reached a level slightly greater than normal on the thirty-ninth postexposure day, and fluctuated slightly above and below the preexposure levels for the remainder of the 60 days.

(c) Total Leucocytes. The total leucocytes decreased rapidly after irradiation. On the second postexposure day the counts were 64 per cent of the preexposure mean count, and by the seventh day they had fallen to 16 per cent. Counts were minimal from the seventh to twenty-first days, during which time they ranged from approximately 7 to 17 per cent of the preexposure mean. After the twenty-first day the leucocytes increased steadily, and when the experiment ended, on the sixtieth postexposure day, the counts were approximately normal.

(d) Platelets. Platelet counts decreased rapidly after the first postexposure week and during the second to third weeks reached minimal levels of approximately 15 to 20 per cent of the preexposure values. After the third week the counts increased slowly and attained approximately normal levels by the sixtieth day.

(e) Erythrocytes and Hemoglobin. Erythrocyte counts decreased slowly during the first 2 weeks following exposure and reached minimal levels of approximately 70 to 72 per cent of the preexposure mean counts during the third week. Thereafter the erythrocytes increased steadily at a slow rate and varied between 80 and 90 per cent of the preexposure mean during the last 4 weeks of the experiment. The hemoglobin trends were closely similar to the erythrocyte trends, and any differences between the two are of doubtful significance.

(f) Reticulocytes. Reticulocytes of dogs were markedly depressed within 24 hr after exposure to 300 r. Minimal counts ranging from 0 to 12 per cent of the mean preexposure levels prevailed from the first to eighteenth postexposure days. Thereafter reticulocytes increased fairly rapidly. Maximum counts approximately three times the preexposure level were attained on the forty-sixth postexposure day. Subsequently the trend of the reticulocytes was toward normal, although counts still ranged considerably above the preirradiation level on the sixtieth day, when the experiment ended.

4.8 Relation Between Adult Cells and Their Precursors. In the acute rat and dog experiments, in which the animals received doses approximating LD_{50}, the relations between the postirradiation trends in the adult cells of the peripheral blood and the trends of their precursors in the bone marrow were studied. The findings in the rat experiment are reported in detail in Chap. 2 but are briefly summarized here since the relations are of interest in considering hematological changes. The studies on dog bone marrow were part of a large program concerned with the possibility of transplanting normal bone marrow to irradiated animals. This work was carried out at the Rochester Project under the direction of P. E. Rekers.

(a) Circulating Neutrophils and Their Precursors in Rats. Absolute neutrophils of the peripheral blood and cells of the myelocytic series of the bone marrow decreased rapidly during the first 2 days following irradiation with 550 r, and both reached minimal levels approximately 3 to 4 days after exposure. The decrease in the peripheral-blood neutrophils was noted approximately 10 hr earlier than the decrease in the myelocytic cells of the bone marrow. However, the marked variation in both counts throughout the experiment minimizes the significance of the difference.

Peripheral-blood neutrophil counts remained markedly depressed until the eleventh to twelfth postexposure days, at which time they showed a definite increase. Counts of the myelocytic series of the bone marrow varied markedly throughout the experiment. The period of minimum counts was not so clearly defined as it was in the peripheral-blood neutrophils, but it extended approximately from the third to fourteenth days. The first definite increase in marrow cells following the depression was noted about the fourteenth day. Both neutrophils of the peripheral blood and cells of the myelocytic series of the bone marrow had reached preexposure levels by the sixth week, when the experiment ended.

The trend of the myelocytic cells of the bone marrow did not follow the trend of the peripheral-blood neutrophils during the brief period of leucocytosis immediately following exposure.

(b) Circulating Erythrocytes and Their Precursors in Rats. During the first 4 days following exposure to 550 r, the erythrocyte counts of the peripheral blood remained relatively stable. By contrast the total nucleated red cell count of the marrow decreased steadily during this period. The downward trend was definite within 15 hr after exposure and continued until approximately the fifth postirradiation day, at which time the marrow nucleated red cells began to increase slowly toward normal levels. Reticulocytes of the peripheral blood decreased during the first 3 days and were minimal from the third to eighth days. Erythrocytes of the peripheral blood began to decrease about the same time that the marrow erythroid cells began to increase. The reticulocytes of the peripheral blood began to increase about the eighth postexposure day and from the sixteenth to twenty-eighth days were approximately five to seven times the preexposure level. By the forty-second day the reticulocytes and mature erythrocytes of the peripheral blood had returned to approximately normal levels, as had the nucleated red cells of the bone marrow.

The major trends of the peripheral-blood cells and their precursors in rats are summarized in Table 3.7.

Table 3.7 — Trends of the Peripheral-blood Cells and Their Precursors in Rats

Cell series	Time after exposure to beginning of definite decrease of counts	Time after exposure to beginning of recovery from depressed counts, days
Bone-marrow myelocytic cells	30 hr	14
Peripheral-blood neutrophils	21 hr	12
Bone-marrow erythroid cells	15 hr	4 – 5
Peripheral-blood reticulocytes	15 min	8
Peripheral-blood mature red cells	5 days	17

(c) Circulating Cells and Their Precursors in Dogs. Histological examination of postexposure rib-marrow sections from dogs irradiated with 300 r indicated that definite hypoplasia was present as early as 2 days after exposure. Both the erythroid and myeloid series were affected. However, all stages of maturation in both series appeared to be present. On the basis of differential counts, the greatest decrease was in the erythroid series, in which depression appeared to be maximal on the second postexposure day. Absolute counts of bone-marrow cells were attempted but proved entirely unsatisfactory, and no significance could be attached to the data obtained. In the peripheral blood a precipitous decrease in lymphocyte counts began within 24 hr after exposure, and both reticulocytes and lymphocytes had reached minimal levels on the second postexposure day. Counts of other leucocytes were decreasing but were not minimal, and mature circulating erythrocytes remained in almost normal numbers.

Hypoplasia of the bone marrow progressed after the second day and reached a maximum on the seventh to ninth days after exposure. Only occasional small nests of cells were apparent in the sections at that time. Although both erythroid and myeloid series were markedly depleted, the depression of the differential count was most extensive in the myeloid series, and the myeloid-to-erythroid ratio was reversed.* Most of the nucleated red cells were erythroblasts. Leucocyte counts in the peripheral blood reached minimal levels at about the same time that marrow hypoplasia became maximal.

Extreme marrow hypoplasia persisted through the twenty-third postexposure day. The first evidence of regeneration was noted in sections obtained on the twenty-eighth day, at which time there was a marked increase in cellularity in both the myeloid and erythroid series. The myeloid-to-erythroid ratio, however, was still reversed, and the erythroid series continued to be represented largely by erythroblasts. Leucocytes of the peripheral blood were beginning to appear in increasing numbers by the twenty-eighth postexposure day. Reticulocytes showed the beginning of a marked upward trend somewhat earlier (see Table 3.8).

Table 3.8 — Trends of the Peripheral-blood Cells and Their Precursors in Dogs

Cell series	Period of maximum depression, days	Beginning of recovery phase, days
Cellularity of bone marrow	7 – 23	24 – 28
Percentage of myeloid cells in bone marrow	4 – 21	24 – 28
Absolute neutrophils in peripheral blood	7 – 21	22
Percentage of nucleated RBC in bone marrow	2 – 3	4
Mature erythrocytes in peripheral blood	14 – 21	23 – 28
Percentage of reticulocytes in peripheral blood	2 – 18	18 – 20

The tendency toward increasing cellularity of the marrow continued, and by the thirty-ninth day the marrow was definitely hyperplastic. The myeloid series of the marrow increased proportionately more than the erythroid series, with the result that the myeloid-to-erythroid ratio reverted to normal approximately 40 days after exposure. Peripheral-blood leucocyte counts were approaching normal levels on the thirty-ninth and fortieth postexposure days and remained fairly stable thereafter. Reticulocytes of the peripheral blood, however, continued to increase and subsequently reached levels well above normal, as has been noted.

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*The normal myeloid-to-erythroid ratio in these animals was established as 1.9 with a range of 1.3 to 2.2.

When the experiment ended on the sixtieth postexposure day, the marrow appeared to have returned to approximately normal activity. The cellularity, judged by histological sections, was essentially within normal limits, and, in general, the differential counts corresponded closely to preirradiation findings.

The relations between the trends of the cells of the peripheral blood and their precursors in the bone marrow of dogs are summarized in Table 3.8. It should be noted that the figures relative to the myeloid and erythroid cells in the bone marrow refer to percentages and that these figures must be considered in relation to the total marrow cellularity in order to obtain an estimate of the absolute change in marrow elements.

5. DISCUSSION

In many respects the complex biological systems, which are inescapably involved in long-term animal experiments, were ideal for testing the feasibility of using hematological changes as an indication of radiation damage. In regard to their extreme complexity, such systems duplicate the conditions under which biological monitoring would have to function and be interpreted.

In this respect the most significant finding is the necessity of relying on statistical analyses in interpreting experimental results. This is best illustrated in the rat experiments in which the dosages used approached the minimal effective dose. In all instances the changes that occurred after exposure to doses ranging from 25 to 5 r were extremely transient. In addition they were small in magnitude and were superimposed on the inherent diurnal variability of the rat leucocyte counts. It was possible only by statistical interpretation to demonstrate the highly significant depression of lymphocytes that occurred in even the lowest-dosage groups.

It would be impossible to attach any significance to changes of similar magnitude under conditions that might be expected to exist if one man were accidentally exposed to radiation in an amount near the minimal effective dose. It is, in fact, quite unlikely that minor transient changes would even be noted since counts would not ordinarily be taken several times during the day in a routine monitoring system. The obvious inadequacy of routine hematological monitoring in situations such as the one postulated above appears even greater in the light of the reasonable assumption that unrecognized subtle changes almost certainly preceded such gross pathology as abnormal blood counts.

The results of the experiments also indicate that hematological examinations are unreliable as criteria for prognosis in individual cases once radiation damage is known to have occurred. This statement is based on the experimental finding that no consistent or convincing difference between leucocyte counts of survivors and nonsurvivors could be demonstrated in the various experiments.

Comparison of trends of bone-marrow cells with trends of peripheral-blood cells following irradiation with doses approximating LD_{50} indicates that changes are equally prompt and that examination of marrow by routine techniques would offer no particular advantage in indicating radiation damage. It is interesting to note that Jacobson and Marks,[1] in studies of the hematological effects of chronic exposure to lower doses of X radiation, observed that animals which developed mild lymphopenia or a moderate reduction in other cellular elements of the peripheral blood showed no histological evidence of abnormality of the blood-forming tissues.

The reticulocytes in the acute experiments appear to be similar to the lymphocytes in the promptness and extent of their depression following irradiation. This finding contrasts sharply with the radioresistance of mature erythrocytes. A comparable relation between reticulocyte and lymphocyte responses was not noted in the chronic irradiation studies reported in Chap. 11. The fairly large error inherent in reticulocyte counting and the absence of true control groups lead to a cautious acceptance of the results. The response has been observed consistently in both dog and rat experiments, however, and is of sufficient magnitude to suggest that it is real and not an artifact. The marked increase in reticulocytes in the peripheral blood just prior to and during the recovery stage of erythrocyte counts may reflect, in part, the restoration of active precursors in larger numbers and, in part, the utilization of stored iron resulting from formerly damaged red cells. The nature of the data in both rat and dog experiments is such, however, that interpretation is necessarily largely speculative.

Certain of the data in these experiments might appear to offer a good basis for estimating the maturation periods and life spans of various cellular elements in the peripheral circulation. For example, the increase in reticulocytes in rats begins on about the eighth postirradiation day, whereas the increase in the erythrocytes does not occur until about the seventeenth day. This might be interpreted as indicating approximately 9 days as the period required for maturation of reticulocytes. Consideration of the myeloid series, however, in which the increase in mature circulating forms appeared to precede the recovery phase of the precursors, indicates that this method of estimating maturation time is not entirely satisfactory. Attempts to estimate the life span of leucocytes or erythrocytes on the basis of the rate of their disappearance from the peripheral blood in irradiated animals involves several assumptions that do not seem capable of satisfactory substantiation at this time. In particular, it must be known beyond reasonable doubt that X-ray damage is strictly limited to precursors and that irradiation neither decreases the viability of mature cells nor in any way alters their utilization within the confines of the vascular system nor affects the extent of transfer of cells to extravascular sites. These assumptions appear as yet to be poorly substantiated in fact, and for this reason further discussion relative to estimates of life span is deferred. However, see reference 2.

6. SUMMARY AND CONCLUSIONS

Rats, dogs, and monkeys have been exposed to single whole-body doses of X radiation. The doses for rats varied from 1500 to 5 r and those for monkeys from 2000 to 300 r. Dogs received 300 r, a dose approximating LD_{50}. Hemograms of all animals were determined at regular intervals for 25 to 60 days following irradiation. Examinations of bone marrow were included in the studies that used doses approximating LD_{50}. Data pertaining to monkeys have been excluded because of extraneous infections that invalidate attempts to relate the observed changes to the radiation received.

The major findings may be summarized as follows:

1. Results of these experiments indicate that routine hematological examinations are inadequate for detecting slight radiation damage in any one individual. Changes following exposure to small doses can be demonstrated only by statistical methods.
2. The results of routine hematological examinations appear to be unreliable as criteria for determining the prognosis following isolated instances of whole-body exposure to X radiation. No consistent or convincing difference between leucocyte counts of survivors and nonsurvivors could be demonstrated in the various experiments.
3. Lymphocytes and reticulocytes are similar in regard to the promptness and magnitude of their depression following single exposures to whole-body radiation. In general, depression of these cells is more marked and occurs earlier than that of other elements of the peripheral blood.
4. The depression of neutrophils occurs slightly later than that of lymphocytes and reticulocytes.

5. Significant depression of erythrocytes and hemoglobin is the last to occur following exposure, and the dose required to produce such depression is much larger than that required to produce other changes. The recovery phase of the erythrocyte trends is characteristically preceded by a marked reticulocytosis.

6. With routine techniques, examination of bone marrow appears to offer no particular advantage in indicating radiation damage since changes are equally prompt in both marrow and peripheral-blood cells.

7. In general, the magnitude of peripheral-blood-cell changes is roughly proportional to the dose for exposures up to approximately LD_{50}. Doses greater than LD_{50} tend to induce the maximum response and, if sufficiently large, prove fatal in a relatively short time.

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Chapter 4*

CALLICREIN AND RADIATION

By Kathryn F. Fink

1. INTRODUCTION

Similarities in the reactions of dogs to radiation and to callicrein suggested the possibility that callicrein may be involved in some way in the biological effects of radiation. Experiments were therefore undertaken to investigate callicrein as a possible agent responsible for some of the biological changes occurring after X radiation.

Callicrein was first reported by Frey and Kraut.[1] It is a heat-labile nondialyzable hypotensive substance found in most mammalian tissues in small concentrations; relatively high concentrations have been found in the urine,[2] pancreas,[3] and saliva.[4] The urinary output in dogs is decreased to about 20 per cent or less following pancreatectomy.[5] Callicrein in the urine is in the active form, whereas that in the pancreas exists in an inactive state but is rapidly activated by contact with the intestinal mucosa, probably by proteolytic enzyme action.[6] Callicrein in the blood serum is also present in the inactive form but may be activated by treatment with acetone, alcohol, ether, acid, heat, papain, or trypsin.[7] There are at least two different callicrein inactivators occurring in mammalian tissues. One is present in the gamma-globulin fraction of serum,[8] and the other has been found in lymph nodes and spleen.[9] They differ in both chemical and physical properties, but incubation of either inactivator with callicrein results in inactivation of the callicrein, probably by the formation of a complex between the callicrein anion and the inactivator cation.[10] The optimum pH for the reaction with the serum inactivator[7] is 7.5 and for the spleen and lymph-node inactivator,[9] 8 to 9. Free callicrein can be released from the inactive complex by acidification or tryptic digestion.[9] Callicrein must be administered intravenously; it is not effective when administered intramuscularly or intraperitoneally.[11]

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*Based on Report MDDC-212 (II-188-5937).

Westerfeld and coworkers[12] recently reported the production of shock in dogs by the administration of 60 mg of callicrein, which is the average amount excreted daily in the urine by a normal man. This quantity of callicrein was fatal to most of the dogs, and the average survival time of the dogs that succumbed was about 5 or 6 hr. Some of the findings they reported were salivation; vomiting; retching; marked intestinal contractions; bloody diarrhea; rise in rectal temperature; marked decrease or cessation in urinary output; hypotension; increased heart rate; decreased cardiac output; plasma loss; anoxia; acidosis; hemoconcentration with increased hemoglobin concentration and number of red blood cells; a marked leucopenia accompanied by a decrease in polymorphonuclear cells, lymphocytes, monocytes, eosinophils, and basophils; a subsequent leucocytosis, if the animal survived long enough, consisting almost entirely of polymorphonuclear cells, whereas other cell types remained below normal; increased nonprotein nitrogen (NPN); increased serum creatine and creatinine; increased serum inorganic phosphate; prolonged clotting time; and death from respiratory failure. Typical post-mortem findings were enlargement of the mesenteric blood vessels, hyperemia of the intestine, infiltration of blood into the lumen of the intestine, and contraction and hardening of the spleen.

Most of the features of callicrein shock are characteristic of animals that have been irradiated. The normal occurrence of callicrein in mammalian tissues, blood, and urine and the normal presence of a serum inactivator and a tissue inactivator in the spleen and lymph nodes are further suggestive of the possibility that callicrein may have a role in the biological changes occurring after irradiation. Perhaps the striking pharmacological effects of callicrein are normally controlled by excretion by the kidney and by inactivation by the serum and tissue inactivators, and radiation upsets the normal regulation by damage to the kidney, lymph nodes, and spleen. Callicrein was prepared from normal human urine by two procedures: The first method consisted in adsorbing the active fraction from the urine on a uranium acetate precipitate, elution with diammonium phosphate, removal of the phosphate with magnesia mixture, dialysis, adsorption on benzoic acid, and removal of the benzoic acid with alcohol-ether.[13] The second method entailed a 60-hr dialysis of the urine against running tap water, absorption of the active fraction on aluminum oxide,* elution with 1N sodium bicarbonate,[14] dialysis, adsorption on benzoic acid, and removal of the benzoic acid with alcohol-ether. Callicrein prepared by adsorption on aluminum oxide was used in the biological experiments reported in this chapter.

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*Merck, prepared according to Brockman.

2. EFFECTS OF A SINGLE CALLICREIN PREPARATION

The uranium acetate preparations were effective in lowering the blood pressure in the dog, and 3 to 5 mg was fatal to 200-g rats within 24 hr after a single intravenous injection. The uranium acetate preparations were not entirely free of uranium and were not so potent biologically as the aluminum oxide preparations. The latter were frequently fatal to 200-g rats within 24 hr after a single intravenous injection of as little as 0.8 mg. In general, if a rat succumbed after a single callicrein injection, the death occurred within 24 hr after that injection.

Bradford N. Craver tried several methods for the possible bioassay of callicrein, but his few experiments were unsuccessful. The addition of 0.5 μg of callicrein to the perfusate in the perfusion of the hind limbs of the frog resulted in no response in two experiments. Two rabbit ears and two rabbit hearts were perfused and were found to be insensitive to 1.0 g of callicrein. It is, of course, possible that, if larger quantities of callicrein had been used, a response from the perfused tissues may have been obtained. Craver also failed to obtain a response with the addition of 5.0 mg of callicrein to isolated cat intestinal strip preparations, which is contrary to results reported by Werle and Flosdorf.[15] No adequate explanation can be given for the lack of response to callicrein with the intestinal strips since the callicrein preparations used were fatal to rats at 1.0-mg levels and were effective hypotensive agents in the dog. Vascular responses of six anesthetized dogs to small amounts of callicrein injected intravenously were studied. The dogs were anesthetized by intravenously administered sodium pentobarbital in a dose of 0.5 grain/kg or less. A carotid arterial, a femoral venous, and a pleural cannula were arranged for recording blood pressure, injecting drugs, and recording respiration, respectively. Responses were recorded by kymograph. Hypotensive effects were observed with doses of 1.0 to 2.0 mg of callicrein. Table 4.1 shows typical results of assays of the same callicrein preparation in four different dogs. It can be seen that the effects of the same preparation in different dogs varied considerably, and repeated injections in the same dog did not produce the same effect either in absolute fall or in percentage fall from the control blood pressure. There was a tendency for the initial injection of callicrein to produce the greatest effect on the blood pressure. The data in the last column of Table 4.1 show that the assay is far from ideal.

It perhaps could be made into a reasonably accurate assay by the use of numerous injections of a standard preparation before and after an injection of an unknown preparation. If the use of a large number of standards resulted in a reliable assay, the preservation of a standard preparation would have to be worked out since the preparations in the purified state seem to lose some activity with time.

Table 4.1 — Assay Results of a Single Callicrein Preparation

Dog No.	Callicrein injected, mg	Drop in blood pressure, mm Hg	Control blood pressure, mm Hg	Fall in blood pressure, %	Fall in blood pressure, %/mg callicrein
313	2.0	93	167	56	28*
	2.0	25	175	14	7
	2.0	41	175	23	12
	2.0	26	170	15	8
	2.0	39	167	23	12
	2.0	14	150	9	5
	2.0	22	141	16	8
	2.0	17	141	12	6
	2.0	17	141	12	6
	2.0	14	131	11	5
	2.0	17	134	13	7
	2.0	27	85	32	16
312	1.0	68	124	55	55*
	1.0	48	125	38	38
	1.0	34	94	36	36
	1.0	34	162	21	21
	1.0	38	156	24	24
	1.0	38	153	25	25
	1.0	22	114	19	19
	1.0	32	112	29	29
305	2.0	52	102	51	25
	1.5	39	93	42	28
315	2.0	26	97	27	13

*The first callicrein injection that the dog had received.

3. HEMATOLOGICAL STUDIES

Callicrein shock was produced in three dogs to determine whether callicrein preparations resulted in the responses reported by Westerfeld.[12] Hematological determinations were made 17 to 20 hr prior to treatment. All dogs were anesthetized with sodium pentobarbital before the injection of callicrein. Dog 83 had carotid, pleural, and femoral cannulae inserted. Both dog 83 and dog 84 received occasion-

Table 4.2 — Hematological Changes* in Dogs After Callicrein Administration

Dog No.	Time,† hr	RBC, 10^6/cu mm	Hgb, g/100 ml	Packed cells, vol. %	Abs. neutrophils/cu mm	Abs. lymphocytes/cu mm	Nucleated RBC, %	Erythroblasts, %	Poikilocytosis	Anisocytosis	Basophilia	WBC/cu mm	Neut., %	Eosinophils, %	Basophils, %	Lymphocytes,	Blast forms, %
83	−20	6.9	14.0	50	10,570	1,044	0	0	0	1+	0	13,050	81	11	0	8	0
	0	6.2	14.7	47	4,716	852	0	0	0	1+	0	6,550	72	15	0	13	0
	1	7.9	17.6	58	613	560	11	12	2+	1+	1+	1,333	46	10	2	42	0
	2	8.4	17.4	55	2,308	780	7	18	0	1+	1+	3,250	71	4	0	24	1
	3	8.0	15.7	59	2,349	406	4	11	1+	1+	1+	2,900	81	3	1	14	1
	4	8.9	18.4	60	3,120	440	8	14	1+	1+	1+	4,000	78	9	1	11	1
84	−17½	7.7	17.9	55	7,100	2,200	0	0	1+	1+	0	10,000	71	7	0	22	0
	0	7.2	16.2	52	8,976	2,640	0	0	0	1+	0	13,200	68	8	3	20	1
	1	8.0	19.8	61	2,790	1,215	3	18	2+	1+	1+	4,500	62	4	5	27	2
	2½	9.0	20.6	64	2,756	1,924	34	22	2+	1+	1+	5,200	53	5	4	37	1
	3	8.5	20.1	66	5,168	1,020	11	9	2+	1+	1+	6,800	76	5	2	15	2
	4	8.6	20.4	65	8,058	1,224	11	8	1+	1+	1+	10,200	79	5	3	12	1
	6	8.4	20.4	65	11,634	1,108	5	3	2+	1+	1+	13,850	84	6	0	8	2
	8	8.8	21.7	65	14,702	1,270	7	2	2+	1+	1+	18,150	81	9	2	7	1
	10	8.8	22.5	66	11,744	1,556	3	3	1+	1+	1+	14,150	83	4	0	11	2
84A	−18	7.4	16.7	56	8,085	1,680	0	0	1+	1+	0	10,500	77	4	0	16	3
	0	7.3	18.2	58	7,688	1,948	0	0	1+	1+	0	10,250	75	1	2	19	3
	1	10.5	20.6		598	1,472	5	19	0	1+	1+	2,300	26	2	3	64	5
	2	9.8	21.1		756	1,647	7	37	1+	1+	1+	2,700	28	1	8	61	2
	3	10.1	21.4		1,144	1,300	16	37	1+	1+	1+	2,600	44	1	3	50	2
	4	10.6	21.5	77	1,862	1,606	31	68	2+	1+	1+	3,650	51	0	2	44	3
	5½	10.5	22.0	78	6,818	7,424	28	53	2+	1+	1+	15,150	45	4	1	49	1

*No monocytes, myelocytes, plasma cells, or basophilic stippling were observed in any of the dogs tested.

†After administration of callicrein.

al heart punctures, and dog 84A received none. After the dogs were anesthetized, a second blood count was taken, and then each animal was injected with 60 mg of callicrein. Dog 83 died 4½ hr after the callicrein was administered; dog 84 died after 10¾ hr; dog 84A died 5½ hr after the injection. Within 3 min after the injections were started, the blood pressure dropped precipitously and remained low. Cramps, retching, salivation, vomiting, and diarrhea were common symptoms. Plasma CO_2 capacity was studied in two of the animals. The values dropped within 1 hr after injection of callicrein and continued to decline until death. The control level for dog 83 was 71 vol. %, and the level ½ hr before death was 40 vol. %. In dog 84 the initial value was 69 vol. %, and the premortal value was 42 vol. %. Obviously, marked acidosis had developed. The hematological findings are given in Table 4.2. All three dogs reacted in a somewhat similar way. In each case the injection of callicrein was followed by a rise in red blood cell count, hemoglobin, and hematocrit and a drop in white blood cell count and absolute neutrophils. A terminal rise in the number of white blood cells and absolute neutrophils occurred in the two animals that survived longer than 4½ hr. A slight fall in the number of absolute lymphocytes occurred after injection of callicrein, and in one of the three animals there was a marked terminal rise. Within 1 hr after the injection of callicrein a considerable number of nucleated red blood cells and erythroblasts were observed. The immature cells were probably forced into the peripheral blood from the bone marrow or some other hematopoietic site as a direct or indirect response to callicrein. They have been observed in the peripheral blood of dogs recovering from radiation but do not appear before about 1 or 2 weeks following radiation. The results obtained with the three dogs were in agreement with those of Westerfeld.[12]

4. ABSORPTION-SPECTRA STUDIES

Absorption spectra on three callicrein preparations were determined in a Beckman spectrophotometer. The results are shown in Fig. 4.1. Preparation 39 was obtained by the uranium acetate method, and preparations 81I and 84II were obtained by the aluminum oxide method. The three preparations were made up in phosphate buffer, pH 7.4, at a concentration of 0.25 mg/ml. The absorption peak occurred at about 260 mμ. The absorption peaks were greater in the aluminum oxide preparations, which may be related to the higher percentage of furfural obtained (Table 4.3) as well as to their greater biological potency. Human urine was pooled and dialyzed for 60 hr, and absorption spectra were determined. The absorption maximum was found to be at about the same wavelength as that for callicrein

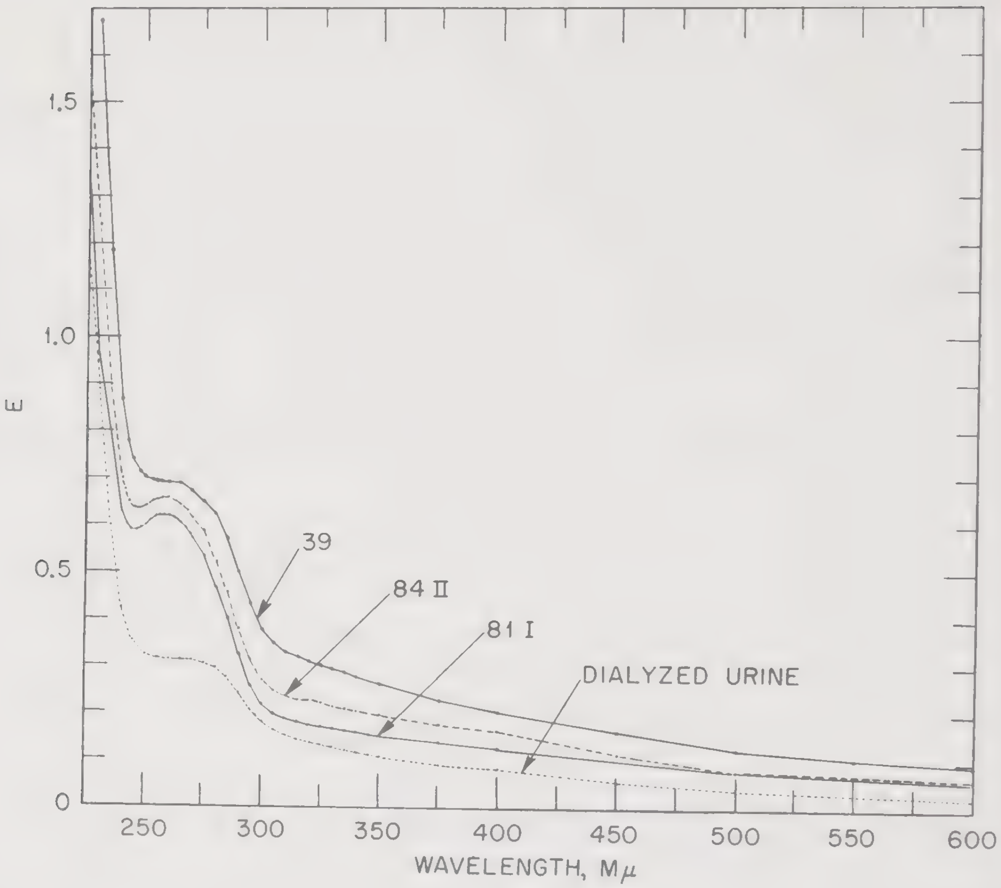

Fig. 4.1—Absorption spectra of callicrein preparations and dialyzed urine.

Table 4.3—Analyses of Callicrein Preparations

Callicrein preparation No.	Method of preparation	Furfural obtained, %	P found, %	N found, %	Ribonucleic acid calculated from furfural,* %	Ribonucleic acid calculated from P,† %
74 I	Al_2O_3	1.66	0.34	11.7	5.7	3.6
74 II	Al_2O_3	1.23	0.41	12.1	4.2	4.3
81 I	Al_2O_3	1.51	0.74	11.9	5.2	7.8
70 II	Al_2O_3	1.04	0.20	12.6	3.6	2.1
70 I	Al_2O_3	1.41	0.39	10.5	4.8	4.1
39	Uranium	0.89	0.37	12.0	3.1	3.9
41	Uranium	0.65	0.24	11.4	2.2	2.5

*Assuming that all the furfural is from ribonucleic acid.
†Assuming that all the phosphorus is in the form of ribonucleic acid.

solutions. The difference in the shape of the callicrein curves and the dialyzed urine curve seemed to indicate that the procedure for preparing callicrein from dialyzed urine resulted in a concentration of a fraction that absorbs ultraviolet light in the region of 250 to 270 mμ. It is to be stressed, however, that evidence is indirect that callicrein alone exhibits an absorption maximum at approximately 260 mμ. It may be that nondialyzable impurities present in the callicrein preparations are responsible for that peak.

The absorption maxima at 260 mμ exhibited by the callicrein preparations, together with the protein nature of callicrein, suggest a nucleoprotein. Several preparations were analyzed for phosphorus using the photocolorimetric procedure of Gomori,[16] for nitrogen by the micro-Kjeldahl method, and for furfural by the acid hydrolysis—steam distillation technique described by Brachet.[17] The results are presented in Table 4.3. The percentage of ribonucleic acid in column 6 was calculated on the assumption that all the furfural was obtained from ribonucleic acid. According to Brachet, ribonucleic acid yields 29.4 per cent furfural, desoxyribonucleic acid yields 0.21 per cent furfural, and glycogen or hexoses yield 3.7 to 3.9 per cent. The low yield of furfural from desoxyribonucleic acid tends to eliminate this type of nucleoprotein as the chief constituent of the callicrein preparations. If the phosphorus is an intrinsic part of the callicrein molecule, glycoproteins are eliminated. The average phosphorus content of 13 other callicrein preparations, not listed in Table 4.3, was 0.46 per cent. The values in column 7 for the percentage of ribonucleic acid, calculated on the basis that all the phosphorus present in the preparation is ribonucleic acid phosphorus, compare fairly well with those in column 6 if the impure state of the callicrein is considered. The nitrogen values are about 25 per cent below theoretical, both for nucleic acids and proteins, but this may be indicative of only a crude preparation. Ribose nucleoproteins have been reported to contain from 5 to 40 per cent ribonucleic acid.[18] The ribose nucleoproteins that have received the most thorough study are the tobacco mosaic virus and its strains. Stanley and Knight[18] determined the phosphorus content of the tobacco mosaic virus and six strains, as well as cucumber viruses 3 and 4, and found the phosphorus content to be from 0.52 to 0.56 per cent, all of which was in the nucleic acid portion of the molecule. The general shape of the absorption curves of callicrein is similar to that of the tobacco mosaic virus.[19] Thus the absorption spectrum, the presence of a small amount of phosphorus, the presence of a furfural-yielding substance, and the protein nature of the material are all in accordance with a ribose nucleoprotein or of a nucleotide associated with a protein.

If the absorption peak at around 260 mμ, which was found with dialyzed urine and the callicrein preparations, is due to callicrein, then by measuring the ultraviolet absorption spectra of dialyzed urine from the same animal before and after irradiation it should be possible to determine the effect of radiation on the excretion of callicrein

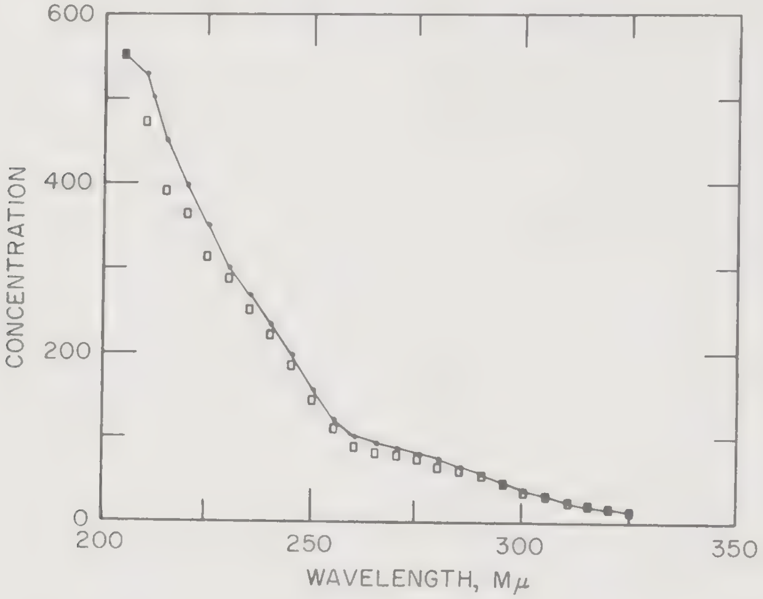

Fig. 4.2—Average daily concentration of ultraviolet-absorbing substances in nondialyzed urine. The concentration units are arbitrary units obtained by multiplying the extinction coefficient by the dilution factor of the urine. •—•, concentration before radiation. □, concentration after radiation.

in the urine. Admittedly, this is an indirect method of approach to the problem, but the biological assays which were attempted for callicrein in nondialyzed urine, dialyzed urine, and callicrein preparations have not been sufficiently reproducible to merit their use for such a problem. Furthermore, the method of isolating the callicrein fraction from urine is not quantitative.

A dog was placed in a metabolism cage, and the urine was collected daily. The volume of urine, the urea concentration, and the absorption spectra of nondialyzed and dialyzed urine were determined daily for 19 days. The dog was then exposed to 150 r of X radiation, and the urine was analyzed on the following 17 days. The average daily concentration of ultraviolet-absorbing substances in nondialyzed urine before and after the animal was irradiated is shown in Fig. 4.2. The two curves are essentially identical, and the same is true when the comparison is made at wavelengths beyond 325 mμ. The average daily concentration of ultraviolet-absorbing substances in the dialyzed urine, shown in Fig. 4.3, likewise was not affected by radiation.

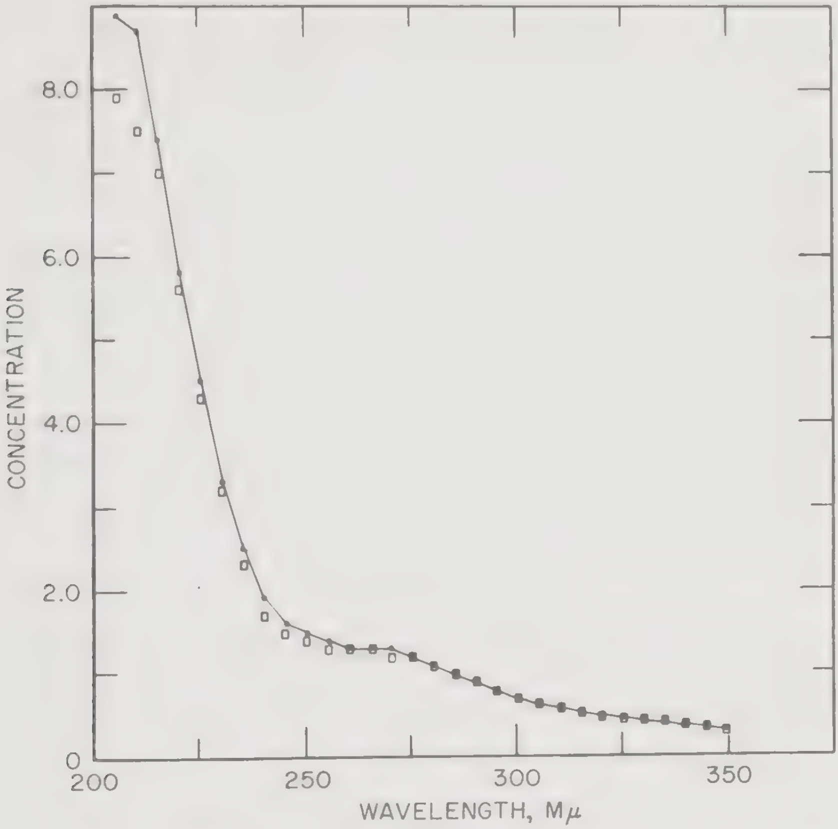

Fig. 4.3—Average daily concentration of ultraviolet-absorbing substances in dialyzed urine. The concentration units are arbitrary units obtained by multiplying the extinction coefficient by the dilution factor of the urine. •—•, concentration before radiation. □, concentration after radiation.

Figure 4.4 shows what percentages of the total ultraviolet-absorbing materials (found in nondialyzed urine) are comprised of nondialyzable ultraviolet-absorbing materials (found in dialyzed urine) at various wavelengths. There was no significant change as a result of radiation. In Figs. 4.5 and 4.6 the average daily excretion of ultraviolet-absorbing substances before radiation was taken as 100, and the excretion after radiation was taken as a percentage of the excretion before exposure. In both dialyzed and nondialyzed urine the concentration of ultraviolet-absorbing materials was about the same before and after radiation, whereas the total amount excreted after radiation was about 50 per cent of the level before radiation. The average daily urinary volume after radiation was 51 per cent of the average volume before radiation. The average daily urea excretion after radiation was 43 per cent of that before radiation, and the average daily urea concentration after radiation was 84 per cent of that before radiation.

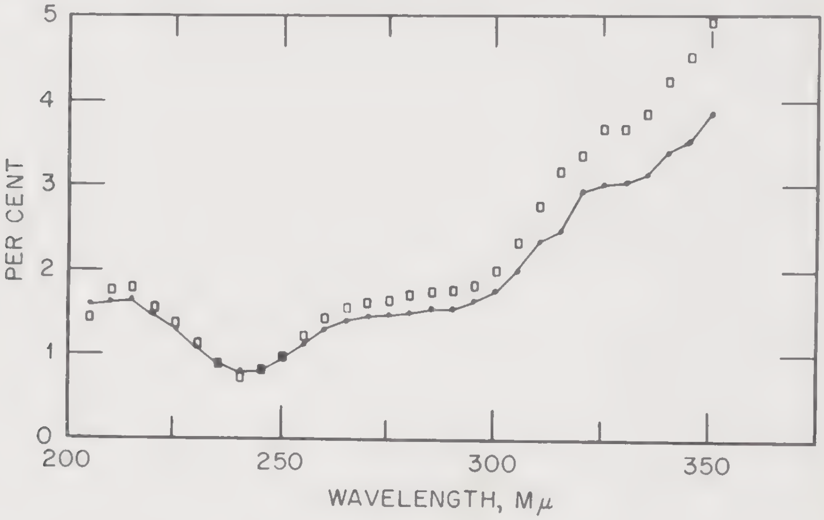

Fig. 4.4—Percentages of the total ultraviolet-absorbing substances in urine comprised of nondialyzable ultraviolet-absorbing substances. •—•, average values before radiation. □, values after radiation.

The results on this single dog, which was exposed to 150 r of X radiation after a control period of 19 days, indicate that the urinary concentrations of urea and ultraviolet-absorbing substances, both dialyzable and nondialyzable, were not much affected by radiation. The total excretion of these urinary products was reduced after radiation to about one-half the normal production, as a consequence of the urine volume being reduced about 50 per cent.

If it is correct that callicrein absorbs ultraviolet light with the maximum occurring at about 260 mμ and if the results of the single dog experiment are typical, some speculations on the metabolism of callicrein in the dog after irradiation can be made. It seems likely that the average concentration of callicrein excreted in the urine was not affected by radiation, but the average total daily excretion of the compound was reduced to about one-half the normal level. It is, of course, conceivable that the concentration of callicrein in the urine was increased (or decreased) if other nondialyzable substances exist

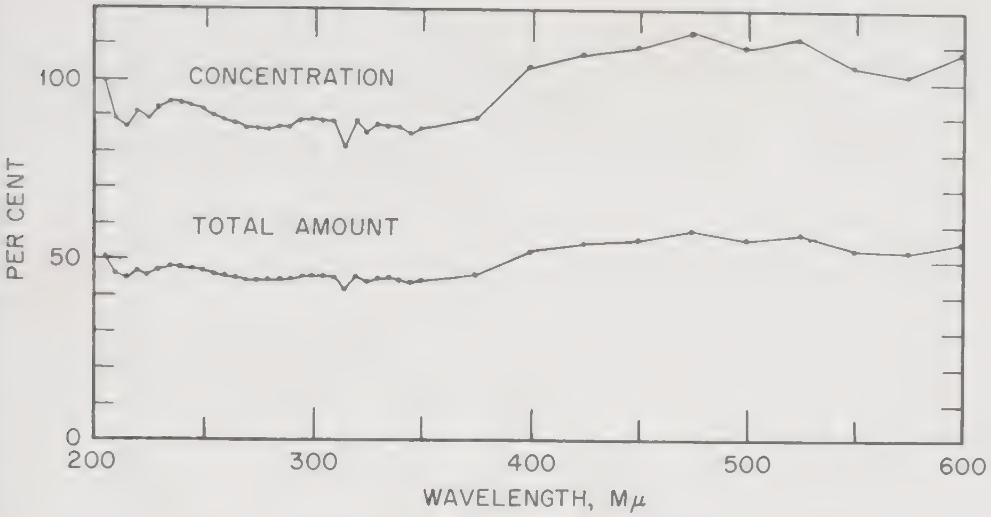

Fig. 4.5—Average daily excretion of ultraviolet-absorbing substances in nondialyzed urine after radiation as a percentage of the average daily values before radiation.

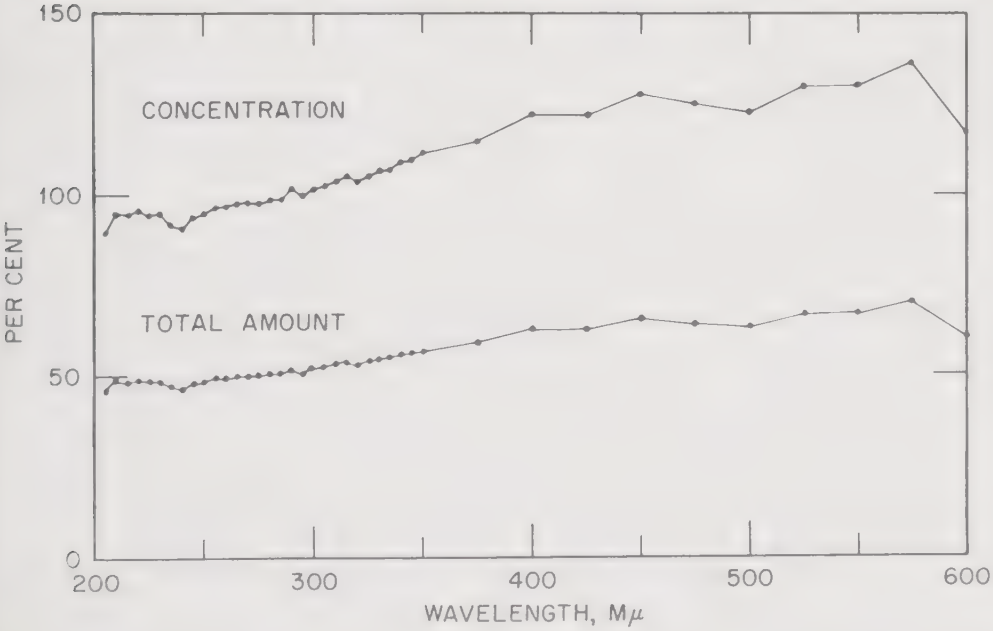

Fig. 4.6—Average daily excretion of ultraviolet-absorbing substances in dialyzed urine after radiation as a percentage of the average daily values before radiation.

in the urine, which absorb at 260 mμ and which were decreased (or increased) after radiation so that a change in concentration of callicrein was masked. The fact that the average daily urea concentration was not much changed after radiation, whereas the average daily total urea excretion was reduced after radiation to about 40 per cent of the control average, makes it seem a little less likely that a change in the concentration of callicrein occurred that was masked by opposite fluctuations in other ultraviolet-absorbing materials that were not dialyzable.

If the callicrein excretion in the urine is reduced to 50 per cent of the normal level after radiation, there are several possible mechanisms by which radiation may have affected the biological handling of the compound:

1. Production of callicrein may have been normal or above normal, but it accumulated in the tissues or blood and was not excreted by the kidneys. If this is the case, the callicrein that accumulated in the body may easily have produced further damage to the animal. A series of blood-transfusion experiments in rabbits has been reported[20] in which about 50 ml of blood was removed from donor rabbits exposed to X radiation (800 and 1000 r) and injected intravenously into normal recipients from which an equal volume of blood had been removed. The results indicated that, at the time of the initial drop in blood pressure with a minimum level at 1½ hr after irradiation, there was some material in the blood that significantly lowered the blood pressure of normal rabbits. Callicrein, a potent hypotensive substance, might be the factor in the blood responsible for this blood-pressure drop.

2. Callicrein formation in the body may have been reduced to about half the usual amount after radiation as a consequence of an abnormal tissue metabolism or a reduced tissue metabolism, and the excretion of the substance as it was formed was normal. Nothing is known about the manner in which callicrein is produced in the animal organism, and it is not known whether callicrein has a normal physiological function such as an enzyme or hormone or whether it is merely a toxic by-product of normal tissue metabolism. There is some indication of a lower tissue metabolism as a result of irradiation. A number of enzyme systems have been reported to be depressed after radiation.[21] In the particular dog studied, the urea excretion over a 17-day period after irradiation was less than half that of the control period. Food and water intake after irradiation is decreased,[22] and that may explain in part the reduced urea excretion and perhaps even the lowered callicrein excretion. It is extremely unlikely, however, that the decreased food intake is the explanation of

the lowered urea excretion for at least the first 2 days after radiation because in starvation the excretion of urea is not diminished significantly for several days after withdrawal of food, whereas in this dog the urea excretion was only 13 per cent of the control level for the first 2 days after radiation.

5. PHOSPHATE-BUFFER STUDIES

Several experiments were performed in which callicrein was given intravenously to rats. The first experiments were designed to determine whether, in two groups of conditioned rats, there is a difference in sensitivity to a large dose of callicrein that was lethal for the majority of assay rats. The two groups of rats were conditioned as follows: one group received daily injections of callicrein in phosphate buffer, and the other received daily injections of phosphate buffer only. The results were then intended to be compared with the results of a test in which the aim was to determine, in two other groups of rats, the difference in sensitivity to a large amount of X radiation that was lethal for the majority of the control rats. The rats for this test were a group of controls and a group of conditioned rats which were exposed daily to small amounts of X radiation. In addition, hematological data were determined.

The first experiment was complicated by the injection of nonsterile solutions. Within 3 hr after the first injection of phosphate buffer and callicrein in phosphate buffer, a reversal of the lymphocyte-neutrophil ratio was found. The phosphate buffer was found to contain B. proteus. When a suspension of B. proteus in beef broth was injected intravenously in rats, a prompt reversal of the lymphocyte-neutrophil ratio occurred. It was felt that the use of nonsterile solutions for injection was the cause of this reversal, although that may not be true. In a later experiment, sterile solutions (shown to be bacteria-free after all syringes were filled) were injected, and the reversal was again found. It is possible that some bacterial contamination may have resulted because no sterile precautions were taken at the site of injection. It is of interest that the data presented by Westerfeld et al.,[12] using sodium bicarbonate as the solvent for the callicrein in their work, show a reversal in the lymphocyte-neutrophil ratio in the two dogs, in which hematological findings were given at 1 hr after callicrein administration.

Two groups of 10 rats each were used in a second experiment. The phosphate buffer, pH 7.4, was passed through a Berkefeld filter, and the callicrein was dissolved in the sterile phosphate buffer each day just before the injections were to be made. The syringes were sterilized, and a separate syringe was used for each rat. The solutions were cultured after all syringes were filled and were shown to be negative. The animals were weighed daily just before injections were made. Blood for hematological study was obtained twice before any injections were made and, on 6 days when injections were made, was obtained between 3 and 4 hr after the injections had been completed.

Table 4.4 — Injection Record and Results

Day	Callicrein injected in 0.5 ml PO_4 buffer, mg	No. of deaths	Wt., g	RBC, 10^6/cu mm	Hgb, g/100 ml	Reticulocytes, %	Platelets, 10^5/cu mm	WBC/cu mm	Neutrophils, %	Lymphocytes, %
					Group I					
−4	Control	0	179	6.7	14.5	4.0	6.4	11,800	24	76
−2	Control	0	187	7.4	15.7	4.5	5.6	14,250	16	83
1	0.4	0	198	7.6	14.2	4.9	5.0	16,200	80	20
2	0.4	0	189							
3	0.4	0	190							
4	0.4	0	192	6.8	13.3	1.5	1.8	28,700	66	33
5	0.4	0	196							
6	0.48	0								
7	0.58	0	201							
8	0.85	0	204	5.9	10.8	5.9	2.8	20,050	68	31
9	0.60	0	203							
10	1.0	0	203	6.0	10.4	9.2	1.7	17,850	65	35
12	2.5*	1	216	5.8	11.5	15.3	3.4	12,000	72	28
13	5.0*	0	213							
15	10.0*	0	224	6.3	11.3	10.9	1.6	9,450	44	56
					Group II					
−4	Control	0	175	6.7	14.3	4.2	4.8	14,350	32	68
−2	Control	0	181	6.8	14.6	4.2	4.7	16,600	29	69
1	0	0	194	7.0	13.9	5.4	5.9	33,250	56	43
2	0	0	195							
3	0	0	198							
4	0	0	199	7.1	13.5	4.4	5.0	28,640	50	49
5	0	0	199							
6	0	0								
7	0	0	202							
8	0	0	207	6.4	13.7	5.6	6.2	27,800	41	59
9	0	0	208							
10	0	0	204	6.8	13.3	5.9	5.9	28,200	51	48
12	2.5*	2	215	7.9	14.6	7.2	4.4	9,750	63	36
13	5.0*	0	203							
15	10.0*	0	208	6.9	12.6	2.0	1.5	14,300	68	32

*Volume injected, 1.0 ml.

A protocol of the experiment has been given in Table 4.4. On the basis of assay results of the callicrein preparation used in this experiment, the 2.5 mg of callicrein injected on the eleventh day should have been fatal to at least half the rats. Only 1 of 10 rats of group I (callicrein) died, and only 2 of 10 rats of group II (phosphate buffer) died after the injection of 2.5 mg of callicrein. On the following day

5 mg of callicrein was given, and 2 days later 10 mg of callicrein was given. No deaths occurred. The results of this experiment seemed to indicate that injections of phosphate buffer and small doses of callicrein protected the rats from a dose of callicrein that should have been lethal. A loss of about 5 per cent of the body weight occurred in the rats in group I within 1 day after the first injection of callicrein, and with continued injections several days elapsed before the weights of the rats in the two groups were equal. Another difference in weight between the two groups was apparent on the thirteenth and fifteenth days. On the basis of weight changes the rats that had been given callicrein earlier were able to withstand the effects of large doses of callicrein better than the rats given only phosphate buffer. There were a number of alterations in the hematological picture in the rats that had received small quantities of callicrein for 10 successive days and subsequently three large doses. On the eighth day the red blood cell count and hemoglobin concentration were lowered and remained at the depressed level over the remainder of the period of observation. The percentage of reticulocytes was low on the fourth day, and subsequent determinations were found to be considerably above normal. The number of platelets was decreased by the fourth day and remained low throughout the experiment. There was an increase in white blood cell count during the period in which small amounts of callicrein were given and a depression after the large quantities were administered. Within 3 hr after the first injection of callicrein, a reversal in the lymphocyte-neutrophil ratio occurred, and it was also found in the subsequent determinations. Hematological changes were also observed in the rats in group II. There was an increase in the percentage of neutrophils and a decrease in the percentage of lymphocytes during the period of phosphate-buffer injections, although the changes were not so pronounced as those found in group I over a corresponding period of time. Following the injection of callicrein the percentage of neutrophils was further increased with a corresponding decrease in the percentage of lymphocytes. The white blood cell count was higher over the period of phosphate-buffer injections and dropped after the callicrein was given. The platelet count showed little change until callicrein was given, and then it was depressed considerably. After the first injection of callicrein there was an increase in percentage of reticulocytes, and a decrease was noted following the third injection. The red blood cell count and hemoglobin were not much affected.

In order to test the theory that previous injections of small amounts of callicrein and phosphate buffer protected the rats from a large (normally lethal) dose of callicrein, another experiment was performed. Eighty rats were sorted at random and divided into three groups of 6 each and three groups of 10 each, and the remainder were used for assays and controls. The details of injection and the results are given in Table 4.5. The results of this experiment show that previous injections of callicrein in phosphate buffer or phosphate buffer alone protect rats against doses of callicrein considerably above the lethal level as determined by assay in rats that had received no previous injections (group I). The rats in groups II and V had also received no previous injections; 4 of 6 of these rats died after the intravenous

Table 4.5 — Injection Record and Results

Group	Day	Callicrein injections,* mg	Number of rats	Deaths	Survivals
I (assay)†		1.9, 2.0, 2.0, 2.3, 2.6, 3.0, 3.75, 3.75, 3.75, 3.75	10	9	1‡
II	1 – 3	None	6		
	4	3.75	6	4	2
III	1 – 3	§	6		
	4	3.75	6	0	6
IV	1	0.6	6		
	2	0.84	6		
	3	0.98	6		
	4	3.75	6	0	6
V	1 – 4	None	12		
	5	7.5	12	12	0
VI	1 – 4	§	12	1¶	
	5	7.5	11	2	9
VII	1	0.6	12		
	2	0.84	12		
	3	0.98	12		
	4	1.88	12		
	5	7.5	12	0	12
VIII (control)	1 – 5	None	16	0	16

*Volume injected when 3.75 mg and 7.5 mg of callicrein were given was 1 ml; volume for all other injections was 0.5 ml.

†Each rat in this group received only one injection (see column 3 for individual injections).

‡The surviving rat in this group was the one that had received an injection of 2.3 mg.

§PO_4 buffer was injected; no callicrein was administered on these days.

¶This death was due to air embolism.

administration of 3.75 mg of callicrein, and all 12 rats in group V died after a single injection of 7.5 mg of callicrein. None of the rats that had received previous injections of callicrein and only 2 of the rats that were given previous injections of phosphate buffer succumbed after the administration of a large dose of callicrein. If it is only the small amounts of callicrein that give protection against a large amount of callicrein, a possible explanation might be that of desensitization of the rats to the protein. No satisfactory explanation can be given at this time for the increased resistance of rats to callicrein afforded by earlier injections of phosphate buffer. Perhaps the buffering capacity of the blood was increased, acidosis did not develop after callicrein, and the inactivation of callicrein by the serum inactivator was at an optimum. It would be desirable to repeat the experiment using another solvent for the intravenous administration of callicrein. Unfortunately the callicrein preparations are only slightly soluble in physiological saline. It is of interest to recall that there was a tendency for the first injection of callicrein to produce the greatest effect on blood pressure in the dogs used for assay of callicrein and also that injections of both phosphate buffer and callicrein in the buffer resulted in a reversal of the neutrophil-lymphocyte ratio in rats.

6. EFFECTS OF CALLICREIN ON X RADIATION

If callicrein is an important factor in deaths from acute X radiation, it seemed worth while to determine whether rats made refractory to callicrein are more resistant to X radiation. The surviving rats in the eight groups described in Table 4.5 were exposed to 750 r of X radiation on the seventh day. All the rats died within 22 days after irradiation. The protection of rats from callicrein by previous injections did not protect the rats from death after irradiation at 750 r.

Another experiment was set up to determine whether previous injections of callicrein would protect rats exposed to 600 r of X radiation. There were 12 rats in each group. No injections of any kind were given one group, and, to the other group, nine injections of callicrein were given over a period of 12 days. Each rat in the injected group received a total of 14.8 mg of callicrein. The rats were then given 600 r of X radiation; there were four deaths in each of the two groups. The injections of callicrein did not influence the mortality.

Four groups of rats were used to determine whether rats first exposed to 600 r could be made refractory to callicrein by several injections of callicrein in quantities that were nonlethal to normal untreated rats and to determine the effect that callicrein injections had on mortality. The details and results of the experiment are summarized in Table 4.6. The intravenous injection of 5.0 mg of callicrein was lethal to about half the rats in group I, which had received no previous callicrein injections. The same quantity of callicrein was not lethal to any of the rats in group III, which were not irradiated but had received small amounts of callicrein on the 4 preceding days. In group II, which was irradiated but not given any callicrein injections, about half the rats succumbed. All the rats in group IV that were irradiated and then given six injections of callicrein died.

Table 4.6 — Effect of Callicrein Injections on Mortality of Radiated Rats

Group	Callicrein injections, mg	Radiation, r	Number of rats	Deaths	Distribution of deaths, days after irradiation
I	5.0, 6th day	None	18	8	All at 6
II	None	600, 1st day	18	10	9, 10, 11, 11, 12, 14, 14, 16, 17, 19
III	0.6, 2d day; 0.8, 3d day	None	18	0	
IV	1.0, 4th day; 1.2, 5th day; 5.0, 6th day	600, 1st day	18	18	2, 4, 4, 6, 6, 8, 8, 8, 9, 9, 9, 9, 9, 10, 11, 11, 12, 16

It appears that the irradiated rats were more sensitive to callicrein since all of them died and since the time elapsing between irradiation and death was shorter than for those rats which were only irradiated. Probably some protection against the large dose of callicrein on the sixth day was afforded the rats in group IV by the four injections of small amounts of callicrein after irradiation since only 2 of the 15 surviving rats in this group died within 24 hr after the injection of 5 mg of callicrein. Judging from past experience, if an injection of callicrein is to be fatal to a rat, the death will occur within 24 hr after the injection. In spite of any protection from callicrein that may have been given 13 of the 18 rats in group IV, the rats died. The callicrein was probably an additional strain to rats already in a stressed condition as a result of the irradiation, and the combination of the two factors was too deleterious to permit the animals to recover.

The experimental work described is not sufficient to determine whether callicrein is a prominent factor in the production of the biological changes which occur after irradiation. Even if the indirect evidence that the output of callicrein in the urine is decreased following irradiation is correct, the knowledge of callicrein at this time is too inadequate to permit interpretation of the finding. Other cases of a low excretion of callicrein in the urine have been reported in infancy, childhood, and senesence[2] and in dogs following pancreatectomy.[5] The observation that the mortality of rats after irradiation is increased by subsequent injections of callicrein may only mean that an already abnormal animal could not withstand the effects of an additional stress. The observation that rats protected from a lethal dose of callicrein by previous injections do not have an increased resistance to irradiation is not conclusive evidence that callicrein is not an important factor in radiation damage since it may be that the inactivators of callicrein are inhibited by irradiation.

The desirable approach to the problem of the relation of callicrein to the biological effects of irradiation is dependent on an accurate determination of callicrein and its inactivators, the equilibrium between callicrein and its inactivators, and a better understanding of the normal role of callicrein in the body.

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Chapter 5*

CROSS-CIRCULATION EXPERIMENTS

PART A. RATE OF UTILIZATION OF CROSS-CIRCULATED PLATELETS IN THE THROMBOPENIC CAT

By John S. Lawrence and William N. Valentine

1. INTRODUCTION

Of all the formed elements of the blood, the platelet has been the most difficult to study. This has been largely due to its extreme lability in shed blood and the consequent technical difficulties associated with handling it. In particular, there has been little or no valid conception of the length of time that the average platelet may be expected to survive in the intact animal. Estimations of the average rate of platelet utilization have been derived almost entirely from the observed rate of regeneration in animals rendered acutely thrombopenic by one or another means—a method the validity of which is open to serious question. In addition to these observations, Duke,[1] in three patients with thrombopenic purpura, was able to make an indirect estimate of platelet life span by noting the duration of improvement in hemostasis following direct transfusion. It is also possible to arrive at a rough estimate of the rate of platelet utilization if the figures of Howell and Donahue[2] on platelet counts made simultaneously from venous and arterial blood are accepted. However, these findings were not confirmed by Fidlar and Waters[3] or Tocantins and Bradshaw.[4] This brief pertinent literature will be reviewed in Sec. 2, but it must be concluded that there is little in the way of concrete data to support more than a conjecture.

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*Part A is based on Report MDDC-217 (II-188-5930), and Part B on Report MDDC-326 (II-188-6029), which have appeared in the published literature [Blood, 2: 40-49 (1947) and 3: 593-611 (1948), respectively].

The object of this part of this chapter is to present data obtained by in vivo studies on the cat relative to the average rate of platelet utilization in that animal. In principle the method has been as follows: A cat is rendered chronically thrombopenic by means of radiation and then cross-circulated by means of carotid-to-carotid anastomoses with a normal animal. After equilibrium has been established, the thrombopenic animal possesses in the neighborhood of half the platelets originally belonging to the normal cat. Each animal is then returned to its own circulation, and the rate of disappearance of the cross-circulated platelets is followed by repeated counts. The methods employed will be discussed in detail in Sec. 3. In effect, therefore, the results are analogous to those obtained by following the rate of disappearance of transfused erythrocytes, the radiated animal in this instance having been rendered incapable of producing more than negligible numbers of platelets. Cross circulation serves merely as a device to give the thrombopenic animal a massive direct platelet-rich transfusion. The platelets never leave the vascular system or make any contact other than with an endothelial surface.

2. REVIEW OF THE LITERATURE

Only isolated estimates of the in vivo rate of utilization of the blood platelet in man or experimental animals appear in the medical literature. The majority of these reports are referred to by Tocantins[4] in his excellent monograph on the mammalian platelet.

The earliest report of which the authors are aware is that of Duke[1] in 1910, who found that the hemorrhagic diathesis in three patients with idiopathic thrombopenia was largely relieved by direct blood transfusion. In one case the platelet count was reportedly raised from 3000 to 123,000 platelets/cu mm and in another from 20,000 to 89,000/cu mm. The transfused platelets had almost completely disappeared within 3 days. Duke concluded that, although these platelets may conceivably have been prematurely destroyed by the disease process, they probably were short-lived bodies whose utilization might be as rapid as one-fourth the total number per day. He also mentioned that experimental results of platelet transfusion in animals rendered thrombopenic by benzol suggested a rapid rate of platelet disappearance. Published details on these experiments could not be found. The following year, Duke[5] reported results obtained by observing the rate of platelet regeneration in dogs made thrombopenic by the removal of blood and its reinjection after defibrination. On the average the rate of regeneration under these circumstances amounted to around one-fifth the entire number in the blood per day.

Firket[6] obtained essentially similar results in animals rendered thrombopenic by both the defibrination technique and saponin injection. Similar rates of regeneration have also been noted by Bedson[7] and Tocantins[8] after thrombopenia induced by the injection of antiplatelet serum. In this connection, however, it is also interesting to note that an increase of 400,000 to 1,000,000 platelets/cu mm in 24 hr has been observed after splenectomy in patients with thrombopenia.[4]

In 1916 Minot and Lee[9] noted marked improvement in the coagulation time of hemophilic patients after transfusion. This improvement lasted for approximately 3 days. Minot and Lee felt that this was probably due to the introduction of normal platelets and that the clinical results were in corroboration of the findings of Duke. Of course, more recent work has indicated that improvement in hemostasis in hemophiliacs after transfusion is not due to introduced platelets per se.

Shouse, Warren, and Whipple[10] noted the sudden disappearance of platelets in dogs 7 to 8 days after they received radiation over the entire skeleton. Since virtually all megakaryocytes were destroyed by the amount of radiation given, it was suggested that the life of the platelet in the peripheral blood might be around 7 to 8 days.

Howell and Donahue[2] in 1937 found arterial blood consistently to contain a larger number of platelets than simultaneously removed samples of venous blood. For this reason and because of evidence interpreted as indicating that platelet production in abundance took place in the lungs, it was concluded that new platelets are added to the blood in the capillary areas of the lungs and that a corresponding destruction of platelets occurs as the blood passes through the capillary areas of the systemic circulation. Using the figures given, it is apparent that this would involve a complete replacement of the entire platelet mass in approximately ten complete circulations of the blood. However, Tocantins and Bradshaw[4] (reported by Tocantins) found inconstant relations between the platelet counts of arterial and venous blood. Fidlar and Waters[3] were likewise unable either to confirm a significant differential in the platelet levels of arterial and venous blood or to obtain satisfactory evidence of important platelet production in the lungs.

In view of the conjectural nature of the rate of platelet utilization in either man or experimental animals as suggested by the scanty evidence available in the medical literature, it appeared desirable to investigate the problem by some direct method in which the actual rate of disappearance of normal platelets could be measured.

3. METHODS

Normal cats were rendered thrombopenic by repeated exposures to known amounts of radiation and then, under nembutal anesthesia, were cross-circulated by means of carotid-to-carotid anastomoses with nonradiated animals. The full details of the method of establishing the cross circulation are given in Sec. 10. For this study it is sufficient to say that platelets traversing the anastomoses have a continuous endothelium-lined pathway in their passage from one animal to the other.

Cross circulation was allowed to function long enough to establish equilibrium, and then each animal was returned to its own circulation. In eight satisfactory experiments of this nature, it was possible to elevate the platelet count of the previously thrombopenic cat by 100,000 to 450,000/cu mm. The rate of disappearance of these cross-circulated platelets was then followed by repeated counts until they had returned to their pre-cross-circulation base line or below.

During the period of developing thrombopenia, which usually was about 10 to 12 days, the platelet count was checked at intervals. No animal was deemed sufficiently thrombopenic for use until the platelet count had fallen to approximately 50,000/cu mm; in some instances it was much lower. Control animals, which received identical radiation preparation but were not cross-circulated, were repeatedly checked to be certain that no significant platelet regeneration was occurring during the period of the experiment. These animals invariably died with gross purpuric manifestations and extremely low platelet counts during the progress of the experiment or, if they lived for the entire period, showed no evidence of platelet regeneration.

All platelet counts were made with National Bureau of Standards certified pipets on blood obtained from the marginal ear vein. Rees-Ecker diluting fluid[11] was first drawn up to the 0.5 mark on a red blood cell pipet. Blood was rapidly drawn up to the 1.0 mark, and the pipet was then filled in the usual manner with more diluting fluid. All counts were invariably made in duplicate and discarded unless satisfactory checks were obtained. Sufficient numbers of duplicate counts were made each day to preclude the possibility of an occasional erroneous count being given undue significance. Results were further checked by examination of Wright's stained cover-slip preparations for the numbers of platelets present. Glassware was scrupulously cleaned, and any preparation rendered unsatisfactory by contact hemolysis of erythrocytes was discarded.

In connection with the cross-circulation experiments, observations were made to determine the physiological variations of platelet counts made by this method on normal cats. In 121 counts made in this manner on 55 normal animals, the average platelet count was found to be 422,000/cu mm. The following percentage distribution was obtained.

Platelet count/cu mm	Percentage distribution
100,000–200,000	0
200,000–300,000	17.4
300,000–400,000	32.2
400,000–500,000	21.5
500,000–600,000	24.0
>600,000	4.9
Total	100.0

Total dosage of radiation varied somewhat from animal to animal, depending on their toleration of the initial exposure and on the satisfactory development of severe thrombopenia. The following factors were kept constant: voltage, 250 kvp; milliamperage, 15; target distance, 22 in. to center of cat; filter, aluminum parabolic plus ½ mm of Cu; half-value layer (HVL), 2.1 mm of Cu; and output, 20 to 25 r/min with minor variations.

In seven of the eight experiments an initial dose of 250 to 350 r of whole-body radiation (usually 250 r) was given. One week later the animal received a second dose of 200 to 300 r (usually 200 to 250 r). Four to five days after the second exposure, a third dose of 100 to 250 r was given (usually 150 r). Total dosage therefore amounted to 650 to 800 r over an 11- to 12-day period. Animals in good condition at this time but satisfactorily thrombopenic were used for cross circulation. Although some animals did not survive the period of preparation, a surprising number of animals were obtained in good condition at the time of operation. In one animal, cat 64, the radiation preparation was considerably more gradual. This animal was used in the first successful experiment when radiation technique for producing chronic thrombopenia was still under development.

Since these animals were also markedly granulocytopenic, it was customary to administer penicillin in saline subcutaneously at intervals throughout the experiment.

In all but one instance the thrombopenic animal was cross-circulated with a normal cat. Cat 254, however, was deliberately cross-circulated with an animal splenectomized about 10 days previously. The splenectomized animal in this case was used because its high platelet count (over 900,000/cu mm) made it a particularly desirable donor.

4. PRESENTATION OF DATA

The data are presented in tabular and graphic forms. Table 5.1 shows the base-line platelet level of each animal prior to cross circulation, the level attained immediately after return to independent circulation, and the platelet levels at approximately 24-hr intervals for 5 days thereafter. It is, of course, impossible to tabulate all the platelet counts performed on each animal. It was customary to perform at least four sets of duplicate platelet counts in the few hours after cross circulation. These counts showed some fluctuation for a few hours, probably incident to vascular readjustments associated with return to independent circulation and recovery from anesthesia. To save space, only the first count after cross circulation is given, and it is reasonably representative. It was also customary to make at least three sets of duplicate counts every day on each animal until the pre-cross-circulation base line had again been reached. Again, data are given only on that count closest to 24 hr, or some multiple thereof, after the time that cross circulation was discontinued. The counts tabulated are in complete agreement with the rest of the data.

Figure 5.1 shows graphically the rate of disappearance of cross-circulated platelets in four of the eight experiments. Each point represents the average of a set of duplicate platelet counts, and the fluctuations mentioned previously can readily be noted. These four experiments were selected for purposes of illustration since the peak platelet level attained varied by approximately equal intervals from around 100,000 to better than 400,000/cu mm.

Table 5.2 indicates the average rate of platelet utilization per cubic millimeter per hour computed for each animal. In view of some fluctuations in the counts soon after cross circulation and in view of an insufficient number of counts to determine the exact time after cross circulation that the animal returned to its preoperative base line, it is obvious that the figures given represent an approximation. The platelet level attained after cross circulation was arbitrarily designated as the first count after return to independent circulation; the time of return to the base line was arbitrarily designated as the time of the first platelet count under 50,000/cu mm.

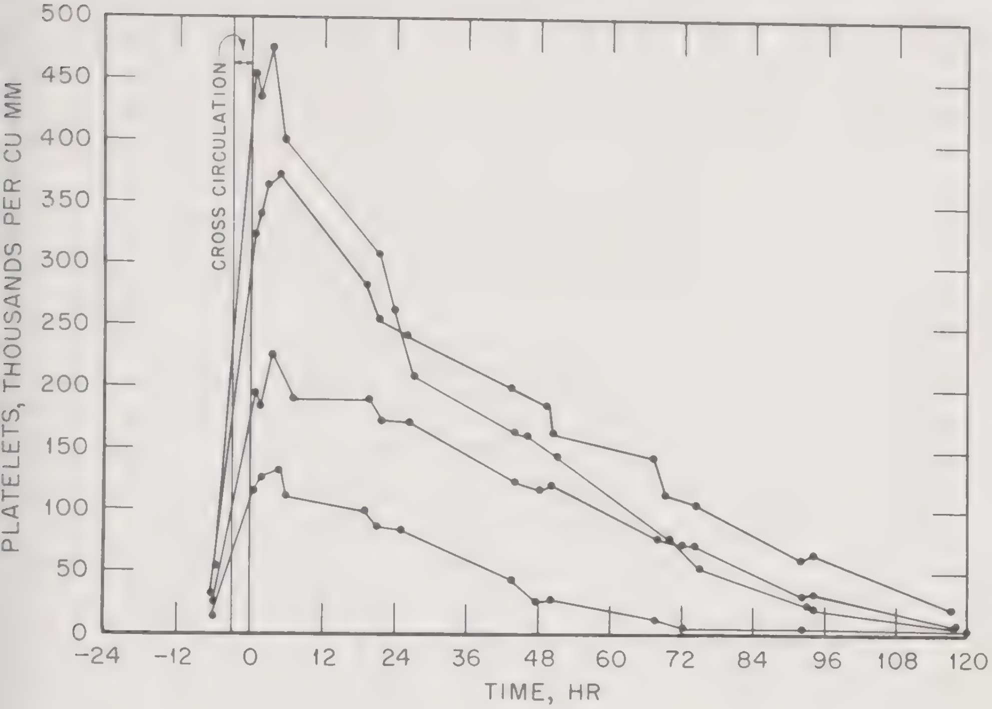
5. ANALYSIS OF RESULTS

From an examination of Table 5.1 it is apparent that no hard and fast rule applicable to every animal can be laid down regarding

Table 5.1 — Platelet Counts of Radiated Thrombopenic Cats Before and at Varying Intervals After Cross Circulation with a Normal Cat

Cat No.	Cross-circulation duration	Count prior to cross circulation, platelets/cu mm	Platelets/cu mm at time indicated after cross circulation					
			First count	20 – 26 hr	44 – 50 hr	68 – 74 hr	92 – 100 hr	114 – 120 hr
254	2 hr, 30 min	52,000	454,000	264,000	163,000	58,000	21,000	9,000
286	3 hr, 22 min	38,000	326,000	246,000	187,000	114,000	67,000	23,000
64	4 hr, 7 min	51,000	272,000	129,000	103,000	64,000	53,000*	39,000*
250	3 hr, 9 min	13,000	117,000	85,000	36,000	6,000	6,000	6,000
252	2 hr, 24 min	26,000	197,000	176,000	119,000	75,000	33,000	7,000
256	2 hr	28,000	248,000	153,000	117,000	42,000	9,000	7,000
222	1 hr, 47 min	15,000	277,000	168,000	119,000	51,000	19,000	12,000
194	2 hr, 55 min	44,000	168,000	136,000	51,000*	29,000	8,000	5,000
	Total	267,000	2,059,000	1,357,000	895,000	439,000	216,000	108,000
	Average	33,375	257,375	169,625	111,875	54,875	27,000	13,500

* Count made slightly outside the specified time limit.



ig. 5.1—Rate of disappearance of cross-circulated platelets in four of the ight experiments. Each point represents the average of duplicate platelet ounts made at the indicated time.

Table 5.2—Rate of Platelet Utilization

Cat No.		Platelets utilized, average number/cu mm/hr
254		4,613
286		2,635
64		2,062
250		1,659
252		1,804
256		2,861
222		2,811
194		1,894
	Total	20,339
	Average	2,542

the rate of platelet utilization. This is, of course, to be expected owing to the fact that platelets when utilized are almost always destroyed. It would, indeed, have been surprising if it had been possible to note a perfectly uniform rate of fall in one individual, much less uniform rates of disappearance in different individuals. Demands for platelets must of necessity vary rather widely in different animals or in the same animal at different times.

Despite lack of complete uniformity from experiment to experiment, certain important generalities or trends show good correlation. In every instance, platelets disappeared from the circulation in a steadily progressive manner with significantly lower counts noted in each successive 24-hr period. In many instances, as in three of four experiments shown in Fig. 5.1, these rates of disappearance roughly paralleled each other, depending on the height of the initial level after cross circulation. Furthermore, there was a general trend for animals receiving relatively slight elevations in platelet count to return to their preoperative base line faster than animals receiving marked elevations in platelet count. The chief exception to this is cat 254, in which the highest platelet level of the group was obtained. In this cat the rate of fall of the cross-circulated platelets was relatively precipitous, and the average rate of platelet utilization was about twice as great as the average for the rest of the group. This animal alone received platelets from a splenectomized donor. Whether the platelets from this donor had been altered in some manner as a result of splenectomy can only be a matter of speculation.

However, the important feature of the presented data is that it took 2 to slightly more than 5 days for cross-circulated platelets to disappear from the recipient. Since preliminary observations on normal cats indicated around 17 per cent had platelet counts between 200,000 and 300,000/cu mm and 32 per cent had platelet counts between 300,000 and 400,000/cu mm, the counts obtained in experimental animals were within the physiological range of about 50 per cent of the normals tested by identical methods. This indicates that, under the conditions of this experiment, the entire platelet mass in the cat requires replacement every 3 to 5 days.

6. DISCUSSION

The data presented constitute the first observations on animals in which rate of platelet utilization has been directly measured. It is rather surprising that the results obtained are in fairly close accord with estimates made indirectly from observations on the regeneration rate of platelets after experimental thrombopenia.[6-8] The rate of regeneration under the intense stimulus of severe platelet depletion would be expected to be a poor index to the normal rate of production (and hence the normal rate of destruction) in the normal animal. The findings are also in rather close agreement with the findings of Duke.[1] The data obtained are not consistent with the observations of Howell and Donahue.[2]

A turnover in the entire number of platelets in the body within a period of 3 to 4 days is probably a minimal estimate, i.e., turnover in the completely normal animal may be slightly slower. The animals employed had received radiation and did have an operative incision to heal. In no case was any infection noted despite close check, and in no animals was there any apparent loss of blood from any source until marked thrombopenia had recurred. However, it cannot be denied that there may have been some increase in demand for platelets in animals under these conditions. There is no evidence that such an increase in demand would be great if it existed at all.

The sequence of events at the time of operation was strong evidence for the role of the platelet in hemostasis. When the neck incision was made in the thrombopenic cat, persistent oozing of blood from traumatized vessels was a major problem. Hemostasis was difficult to obtain, and often oozing persisted to some extent despite all efforts. However, very shortly after cross circulation was established, all oozing ceased spontaneously. The area at the closure was invariably dry, and no difficulty was experienced with bleeding from the wound after operation. It was only 3 to 4 days later, when the platelet count was again very low, that purpuric manifestations recurred. These became progressively worse and were often the cause of death within the following few days.

Care has been taken to refer only to rate of platelet utilization rather than to platelet life span. Since the platelet, unlike the erythrocyte, is destroyed when used, data on the rate of disappearance of transfused platelets are evidence mainly of platelet demands. They disclose nothing as to the length of time that a platelet might survive without spontaneously disintegrating if there were no demands for its use.

In the light of the experiments reported here, the observations of Duke[1] in human beings deserve some further comment. If they could be substantiated, massive direct transfusion would certainly be of importance in assisting patients with idiopathic thrombopenic purpura over critical periods of bleeding or in preparing them for splenectomy. Significant improvement in hemostasis would warrant the use of the more inconvenient direct transfusion rather than the customary method of transfusing stored blood in which platelets are rapidly destroyed. In the experiments reported by Duke, neither the exact amount of blood transfused nor the details of the direct method employed are indicated. However, in this laboratory a direct transfusion of 2000 cc has recently been given within 24 hr to a severely thrombopenic patient with aplastic anemia (platelet count below 10,000/cu mm). The multiple-syringe method was used, and 1500 cc of the transfused blood was given within a 3-hr period. The platelet count was never detectably raised, and no improvement in purpuric manifestations occurred. Bleeding from the nose, which was present before transfusion, continued unabated afterward. The failure to obtain any elevation of platelet count or improvement in hemostasis is unexplained. Experiments are contemplated to determine if possible what happens to the blood platelet during the brief period outside the body during a direct transfusion. It should be emphasized, however, that direct-transfusion methods currently in use are not comparable to the continuous endothelial anastomoses of the animal experiments.

One experiment, of course, proves nothing, and more trials of a similar nature employing other types of direct-transfusion apparatus will have to be made. Nevertheless, the nature of the results brings up the question whether the large elevations in platelet counts reported by Duke following direct transfusion of three patients might, in reality, be coincidental and part of the cyclic variations that are well recognized in idiopathic purpura. The question is worthy of further investigation. If donor blood could be handled in synthetic apparatus that would have no more effect on the platelet than the vascular endothelium in animal experiments, perhaps significant elevations of platelet counts could be obtained.

7. SUMMARY

The rate of utilization of blood platelets in radiated thrombopenic cats was measured directly. Thrombopenic animals, which were incapable of significant platelet regeneration, were cross-circulated by means of carotid-to-carotid anastomoses with normal animals. After return to independent circulation the rate of disappearance of cross-circulated platelets was measured by periodic counts.

By this method it was possible to elevate the platelet count from 100,000 to around 400,000/cu mm. The highest count obtained followed cross circulation of a thrombopenic animal with a splenectomized animal. In most instances the platelet level attained was within the physiological range of that found for normal cats by the same method.

The cross-circulated platelets gradually disappeared from the circulation over a 2- to a slightly more than 4-day period. Therefore under the conditions of this experiment the entire platelet mass would have to be replaced every 2 to 5 days. The same figures probably apply within narrow limits to the normal cat.

The average rate of platelet utilization was approximately 2500/cu mm/hr. In seven of eight experiments the rate of disappearance varied from about 1600 to about 2800/cu mm/hr. In the experiment using a splenectomized donor, the rate of disappearance was about twice the average for the rest of the group.

Attention is called to possible therapeutic implications of these findings in idiopathic thrombopenic purpura.

PART B. IS THERE AN "INDIRECT" EFFECT OF RADIATION?

By John S. Lawrence, William N. Valentine, and Andrew H. Dowdy

8. INTRODUCTION

Since the discovery of the X ray by Roentgen, medical science has shown much interest in elucidating its mode of action. The problem is by no means completely solved. Certain facts, particularly those relating to the results obtained when tissues are subjected to direct radiation, are well established and widely accepted. For example, that an aplasia of bone marrow results from direct widespread radiation in large doses must be regarded as beyond dispute.

However, there are other observations, both clinical and experimental in nature, which are poorly understood and highly controversial. These relate particularly to changes in tissues far removed from the direct site of radiation and have led to the postulation of an indirect action of X rays. For example, many roentgenologists have privately observed and others have publicly reported marked involution of lymph nodes involved in a lymphomatous or metastatic process when radiation was given to other areas sufficiently remote to have precluded any direct exposure of the former. Equally well known are the marked reduction in the blood count of leukemic patients treated by X ray and the leucopenia which frequently develops in similarly treated nonleukemic individuals, even when only very small areas of marrow are subjected to the effects of direct radiation. These effects, at least in superficial appearance, are similar to those well established for direct radiation. The result has been a voluminous amount of interesting but confusing literature reviewed in Sec. 9.

The possibility of an indirect action of X rays is the subject of this part of this chapter. The concept of indirect action requires further definition. In an organism all of whose cells are bathed in a continuous circulating fluid medium and which is interlaced with an intricate network of nervous tissue, it is inconceivable that an agent such as X radiation, known to be capable of creating extensive destruction of certain cells, should be without an indirect effect. It is well established that there are indirect effects from a thermal burn or mechanical crush. Yet, at least in the case of crush injuries, the only action directly attributable to the causative agent ceases at the time the trauma is discontinued. What occurs in the organism after that must be regarded as strictly nonspecific, even though these events may be of a highly serious nature at times. Therefore this study is limited to an effort to demonstrate indirect effects peculiar to radiation. The pertinent question is whether or not radiation results in the formation of specific substances (not merely those liberated by nonspecific cellular injury or death) which, when transported to distant areas, produce a characteristic indirect effect.

If such distant indirect effects exist, it seems reasonable that the agents mediating them will, in all probability, be transmitted to their site of action by means of the circulation. The experimental approach has therefore been to arrange for the direct passage of a very large volume of blood for a period of several hours from a radiated to a normal animal and then subsequently to observe the normal animal for evidence of changes that might be interpreted as indirect radiation effects. Cross circulation by means of carotid-to-carotid anastomoses was found to be satisfactory. Parabiosis, in which small circulatory connections undoubtedly exist between the two animals, was felt to be disadvantageous because only small amounts of blood cross-circulate per unit of time. The methods and techniques employed to establish cross circulation are described in detail in Sec. 10. Important corollary principles are that the radiated animal must receive a relatively large acute dose of radiation in order that any indirect effect present may be made more manifest and that sufficient experiments must be performed to cover all time intervals up to at least a few days after radiation of the one animal. Significant changes in the leucocyte and lymphocyte picture in the normal animal following cross circulation have been selected as the criteria of an indirect effect in these investigations. These criteria were selected because these elements have been shown to be two of the most sensitive indicators of damage by radiation and because changes in these elements and their precursors have been repeatedly mentioned by others attributing indirect effects to radiation.

9. REVIEW OF THE LITERATURE

An appreciable amount of medical literature has accumulated concerning the indirect effects of X rays. Particularly in the early 1900's many contributions to the subject appeared. The German investigators were especially active in this field. Much of this early literature is contradictory but is of great historical value. Dosage of radiation administered was in most instances not accurately known, and the quality of radiation employed varied widely from investigator to investigator. Roentgenology was in its infancy, and technical factors were poorly understood and poorly controlled. The field is not one that readily lends itself to experimental investigation, and even with vastly increased knowledge and improved technical control the same general arguments prevailing soon after the turn of the century are still being debated in the literature of the past 20 years.

Broadly these investigations can be grouped into four main types:

1. The demonstration of or the failure to demonstrate specific toxins (most frequently "leucotoxins") in the serum of patients or experimental animals exposed to radiation. Both in vivo and in vitro studies of this nature are reported.
2. The demonstration of or the failure to demonstrate significant histological changes in tissues ordinarily sensitive to X rays following radiation of some site remote from the tissues studied.
3. The clinical demonstration of significant involution of susceptible tissues far removed from the point of application of direct radiation.
4. A group of miscellaneous investigations chiefly concerned with demonstration of a wide variety of biochemical abnormalities occurring after radiation and purported to be the mediators of an indirect effect.

Efforts to demonstrate that a specific toxin developed as a result of radiation have been particularly numerous. Linser and Helber[12] found that a leucotoxin was produced in the blood of organisms exposed to X rays. This leucotoxin, when injected into other animals, destroyed the circulating leucocytes and, when added to animal exudates containing leucocytes, caused loss of motion of and degeneration of the cells. Curschmann and Gaupp[13] in 1905 injected rabbits with serum taken before and after radiation therapy from a patient with lymphatic leukemia. A few hours after injection, Curschmann and Gaupp observed, in the injected animals, leucopenia that was not present in control animals. They concluded that this was due to the development of a specific leucotoxin that could also be demonstrated as capable of destroying human leucocytes in vitro. They presented evidence suggesting that such a leucotoxin was inactivated by heat (60°C for ½ hr). The following year Klieneberger and Zoeppritz[14] were unable in their investigations to demonstrate any consistently toxic action of radiated serum on leucocytes in vitro in regard to either cellular disintegration or influence on amoeboid activity. In addition, no constant leucopenic effect was observed after injection of radiated serum into rabbits. Klieneberger and Zoeppritz concluded that no toxin could be demonstrated as a result of radiation. Milchner and Wolff[15] observed some decrease in leucocytes in animals after the injection of 5 cc of serum from a radiated animal of the same species. They also noted some degree of leucopenia when material from a radiated spleen was injected, whereas injections of normal spleen resulted in leucocytosis. However, they felt that their results were not of sufficiently concrete nature to be strong evidence for the presence of a leucotoxin, particularly in view of the wide physiological variability in the leucocyte counts of the experimental animals used. Benjamin and coworkers,[16] after radiating the ears of rabbits with large doses while shielding the remainder of the animal, found leucocytosis and lymphopenia occurring, but the blood picture returned to normal within 24 hr. They claimed to have found increased choline formation in animals after intense radiation and to have noted that this corresponded to the period of leucocytosis. The investigations of Capps and Smith[17] were interpreted as supporting the concept of a leucotoxin. These men found that a leucopenia resulted from the injection of a few cubic centimeters of radiated serum from one animal into the abdominal wall of another. This serum was also said to cause abnormal destruction of leucocytes in hanging-drops preparations. Harris[18] concurred with this view, although he presented no experimental evidence of his own.

In 1918 Dorn[19] reported that testicular and ovarian atrophy and leucopenia of considerable duration resulted from the injection of enzytol, a borate of choline. Dorn felt that the effects of radiation were reproduced exactly by this substance and that this offered strong support to the concept of choline as the toxic cause of radiation effects. Walterhofer[20] likewise considered indirect effects of radiation to result from some toxin, possibly choline. In 1922, Billings,[21] in discussing his experiences with the treatment of leukemia by X ray, stated that the application of radiation to any cutaneous surface resulted in the appearance of a leucolytic substance in the blood. The serum of a treated patient dissolved white blood cells in vitro, whereas the serum of an untreated patient had no such effect. In the same year, Murphy, Liu, and Sturm[22] were forced to conclude that their experiments failed to show any evidence of the presence of a lymphotoxin, even when exposures large enough to effect almost complete destruction of lymphoid tissue were employed. Four years later Zacherl,[23] as a result of experiments with radiation of single and parabiotic rats, felt that some toxic substance capable of causing sickness and death of the animals must have been produced. Szilárd[24] in 1927 could demonstrate no autolytic effect on leucocytes in the serum of radiated leukemic patients, nor could any such autolysin be demonstrated by complement fixation tests. He could find no increase in choline in the blood or urine of leukemic patients under X-ray therapy. He postulated, nevertheless, that perhaps electrons circulating in the blood exerted a deleterious effect on distant sensitive cells. Strumia's studies[25,26] led him to make the conclusion in 1929 that

> ... radium emanations, as such, are carried by the blood or that they act through the production of leucotoxins, which are produced by the direct effect of the radium emanations upon the white cells of the blood in superficial vessels, or perhaps upon fixed cells of the reticuloendothelial system. ... the direct action of radiation upon hemopoietic foci is altogether unimportant. In all cases the blood acts as a carrier of radiations, either as such or modified, to the hemopoietic foci where their main action takes place.

Woenckhaus[27] felt that leucopenia observed in normal rats after injection of serum from strongly radiated rats was due to products of proteolysis in the injected serum. However, he also found that, when one member of a parabiotic pair was radiated and the other shielded, a leucocytosis and not leucopenia occurred in the nonradiated parabiont. In 1932, Zwerg[28] heavily radiated the skin flaps of small laboratory animals. The remainder of the animal was shielded; yet, if the pedicles connecting the skin flap to the animals remained intact, the animals invariably died in 2 to 9 days and exhibited varying degrees of leucopenia and lymphopenia, but not of the order of magnitude always seen in animals that have received enough generalized radiation to result in death. Zwerg concluded that the effect of X rays on the white blood cell picture is not a direct action on the blood-forming organs and probably not on the circulating blood. Rather, he believed, the decomposition of cells gives rise to toxins that in turn damage the hematopoietic system.

Macht[29,30] in more recent years has attacked the problem by phytopharmacological methods, examining blood and serum obtained both before and after radiation from a variety of animals. Briefly, his method consists in studying the growth of roots of Lupinus albus seedlings in standard physiological solutions compared with the root growth of similar seedlings in similar solutions to which blood or serum had been added. Macht concluded that there is a toxic substance present in radiated but not in normal serum and that it reaches its maximum titer 24 to 48 hr after treatment and disappears in a few days.

Osgood[31] in 1942 conducted in vitro experiments designed to determine if indirect radiation effects on cells of bone marrow could be demonstrated. He set up four human-marrow cultures identical in every respect except that one received no radiation. In a second culture both cells and medium (35 per cent human serum and 65 per cent balanced salt solution) were radiated; in another, only the medium was radiated; and, in the last, only the cells were radiated. Quantitative hematological studies indicated no indirect action of radiation on marrow cells suspended in the radiated medium.

A number of histological studies have also been made in which tissue remote from the direct site of radiation has been examined for evidence of indirect damage. Nakahara and Murphy[32] found identical changes in both deep and superficial lymphoid tissue following small doses of soft X rays. Since 96.8 per cent of the rays did not penetrate beyond a depth of 1 cm, it was felt that these changes in deep tissue could not be the result of direct action by the rays. Jolly and Ferroux,[33] in histological studies made on lymphatic structures outside the field of direct radiation, were of the opinion that there was grave doubt that the slight changes noted were due to an indirect toxic action of radiation. Rather it was their inclination to attribute such slight changes that might exist to diffused or secondary radiation. Akaiwa and Takeshima[34] exposed popliteal lymph nodes of rabbits to various amounts of radiation, leaving one side as a nonradiated control. The changes in lymphoid tissue on both sides were noted at a variety of intervals after radiation. They concluded that, in the control nodes, histological changes identical with those in the radiated nodes occurred but that the reaction was much less intense and much slower on the nonradiated side. In 1940, Hsu and Ma[35] radiated one femur only of rats with doses varying from 1000 r over a 4-day period to 5000 r over a 40-day period. The animals were sacrificed on the day after the course of radiation was complete, and the bone marrow of radiated and nonradiated femurs, the submaxillary lymph nodes, and the spleen of each were examined. In the nonradiated hematopoietic tissues, changes of a hyperplastic nature, interpreted as compensatory, were noted. Le Blond and Segal[36,37] found that large doses of X rays produced secondary changes in well-shielded organs that were far distant from the point of impact. These changes consisted of constant thymus atrophy, generalized lymphatic atrophy, adrenal hypertrophy, and, frequently, fatty infiltration of the liver and ulcerations of the stomach. Such lesions were considered part of a general intoxication following radiation and were felt to be similar to those described by Selye as occurring in the nonspecific "alarm reaction" developing after the application of a wide variety of injurious stimuli. It is interesting (as has been noted with other noxious agents capable of eliciting an alarm reaction) that thymus and lymphatic atrophy were suppressed by adrenalectomy but that the gastric lesions and general lethal effects of the rays increased. Barnes and Furth[38] in 1943, using single and parabiotic mice, reported an extensive series of investigations. They examined bone marrow, lymph nodes, and spleen in unexposed areas of mice radiated with from 400 to 7000 r. Some slight histological changes were observed, but these were regarded as probably the result of the products of damaged tissue transported by means of the circulation. The degree of change depended on the dose of radiation and probably on the volume of tissue radiated. Barnes and Furth also observed similar histological changes in mice, burned under anesthesia with the actual cautery over the chest and abdomen, and in the lymph nodes of two mice dying of iodoacetic acid poisoning. In addition, the effect on the hematopoietic tissue and lymph nodes of normal mice whose parabionts had been subjected to radiation was studied. Histological changes similar to but much less extensive than those found in the radiated animal were observed in the tissues of the nonradiated parabiont. These were considered nonspecific. Denstad[39] reported on a large number of examinations of bone marrow and peripheral blood in patients who were being treated with X rays. Control specimens were obtained before treatment. The majority of the patients were suffering from malignant disease, and the X-ray dosages, although variable, were, for the most part, cancericidal in magnitude. Marrow specimens were obtained at points a considerable distance from sites exposed to direct radiation. Twenty-eight patients developed no granulocytopenia (below 3000 granulocytes/cu mm). In these there was no abnormality of nonradiated marrow in regard to either total quantity of cells or differential values. In 20 patients the peripheral-blood granulocytes fell below 2300/cu mm. In these cases the cellularity of the nonradiated marrow remained normal, but adult polymorphonuclear leucocytes in the marrow decreased from an average of 16 per cent in the controls to 6.5 per cent in granulocytopenic groups. The promyelocytes were questionably increased. For these reasons Denstad concluded that there is in certain patients some degree of maturation inhibition in myelopoiesis and that this is probably due to some toxic or anaphylactic factor. He considers this to be an indirect effect of X and radium rays on hematopoiesis and suggests that it may occur more readily in patients who are in poor condition.

Although many roentgenologists have observed phenomena suggestive of an indirect radiation effect, relatively few have seen fit to publish these clinical observations. However, Langer[40] has stated that he has observed indirect radiation effects on several occasions. He cites the case of a boy treated with X rays for verruca simplex of both hands. Although only one hand was treated, the warts gradually disappeared from the untreated hand. He refers also to the case reported by Baensch in 1922 of a patient with primary carcinoma of the breast, in whom regional nodes disappeared even though shielded with lead rubber during radiation. Scott[41] has reported to have frequently observed, in cases where large areas were radiated, the disappearance of lymphosarcomatous glands in regions receiving no radiation. In one case he states that this was manifested within ½ hr after therapy.

In addition to the above a miscellany of diverse mechanisms have been postulated as the mediators of indirect radiation effects. In 1905 Musser and Edsall[42] concluded that the effect of X rays is not direct but is dependent on a reaction of the body subsequent to exposure. They felt that the increased catalysis observed after radiation was a response in the form of an increased fermentative process and concluded that favorable response to X-ray therapy could result only when the body was capable of such response. Demiéville[43] concluded that an indirect effect of radiation, probably conditioned through decomposition products, was exhibited in the blood-forming organs. Petersen and Saelhof[44] in 1921 demonstrated increased serum titers of several enzymes after radiation and speculated whether some remote effects of radiation may not be due to enzyme mobilization. Hussey[45] found an uncompensated alkali excess in rabbits exposed to X rays and suggested that alteration in acid-base balance may be an important factor in X-ray action. Opitz[46] suggested that toxins produced by X rays may be agglutinated by tumor cells, thus impairing tumor growth. Rahm and Koose[47] assumed that chemical changes are initiated in the dead interstitial substances of the body and that these may be responsible for the end effects observed. Von Pannewitz[48] observed acidosis followed by slowly increasing alkalosis that persisted for several days after radiation. Kluge and Zwerg[49] have suggested that mobilization of hormones, particularly those of the hypophysis, are important in producing the observed effects of radiation. Very recently Selye,[50] in an extensive review of general adaptation phenomena, has pointed out that many of the metabolic changes described after radiation such as hyperglycemia, decrease in blood cholesterol, increase in ketone bodies in the blood, elevation of the nonprotein nitrogen (NPN), and disturbances in acid-base balance are seen with equal frequency as a response to many injurious stimuli such as traumatic shock, exposure to cold, burns, drugs, and solar rays. He believes that such things represent nonspecific systemic phenomena elicited by injurious stimuli to which the organism is qualitatively or quantitatively not adapted.

From the foregoing discussion it is readily apparent that the literature does not support definitely either side of the question of whether or not there is an indirect effect of radiation.

10. EXPERIMENTAL TECHNIQUE AND METHODS

The experimental animal employed was the cat, and the chief experimental device was cross circulation by means of carotid-to-carotid anastomoses. In each of the 26 successful cross-circulation experiments performed in this study, a normal animal was cross-circulated with a radiated animal, which received its radiation either during the actual time of cross circulation or at varying intervals prior to its establishment. A control group in which normal cats were cross-circulated had already been established in the laboratory in connection with experiments previously reported.[51]

The technique of establishing cross circulation was as follows: The animals were anesthetized with nembutal administered intraperitoneally. The operative site was prepared, and the carotid artery on one side of each cat was isolated and dissected free. A small rubber-covered clamp was placed at the proximal and distal ends of the freed vessel, and the artery was divided midway between the two clamps. Both segments were then washed free of blood with isotonic citrated saline solution. In each animal the proximal arterial segment was cannulated by threading it through a monel cannula, everting the cut end of the vessel over the outside of the cannula, and tying with fine 5-0 catgut. The two animals were then brought together in such a way that the proximal arterial segment of each was directly adjacent to the distal segment of the other. They were supported in this position by small sandbags. A bed for the anastomoses was readily formed by suturing together the strap muscles of the two animals. Following this, the distal arterial segments were drawn over the outside of the cannulated proximal segments and tied about the cannula. Clamps were then released, and cross circulation was allowed from 2 to 10 hr. The cross anastomoses thus established afforded a continuous endothelial pathway by which the blood from each cat supplied one side of the head of its partner and vice versa. Figure 5.2 is a diagram of the method of anastomosis.

The volume of blood traversing an anastomosis of this type will, if uncompensated, result in the exsanguination of an animal within a few minutes. At the conclusion of the period of cross circulation, the patency of each connection was always tested in situ by reapplying the clamps, dividing the anastomoses distal to each cannula, and releasing the clamps for a brief instant.

Prior to an experiment, control hematological studies consisting in hemoglobin determinations and a total and a differential white blood cell count were made. Only clinically well animals showing no gross hematological abnormalities were used. During the period of cross circulation, leucocyte counts and smears were made at approximately 1-hr intervals. In each instance, blood was obtained from the marginal ear vein. The hematological follow-up of the nonradiated animal consisted in a minimum of daily (except Sunday) total and differential white blood cell counts for a period of 2 weeks and similar studies made every other day for a period of approximately an additional 2 weeks. All counts were made with National Bureau of Standards certified pipets. All blood smears were made by the cover-slip method and stained with Wright's stain.

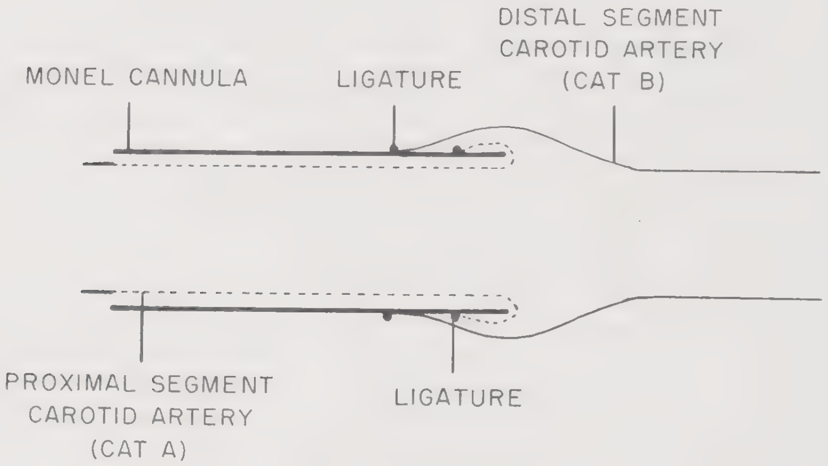


Fig. 5.2—Diagram of end-to-end carotid-to-carotid anastomoses of the type used in these experiments.

The radiation to which one member of each cross-circulated pair was subjected was kept constant and was subject to the following factors: total dosage, 1500 r of whole-body radiation; voltage, 250 kvp; milliamperage, 15; target distance, 22 in. to center of cat; filter, aluminum parabolic plus ½ mm of Cu; HVL, 2.1 mm of Cu; and rate of administration, approximately 25 r/min with slight variation. This amount of radiation (1500 r) invariably results in the death of cats within 4 to 7 days.

In the majority of experiments, one animal was radiated at some interval prior to cross circulation. In seven experiments, however, cross circulation was first established, and then one animal was radiated while the other was shielded. Shielding was accomplished by means of an appropriately shaped wooden frame covered with ¼ in. of lead plate. An ionization chamber placed under the frame recorded the small amount of scatter radiation that escaped the shielding. This scatter varied in different experiments from a minimum of 20 to a maximum of 43 r.

It was customary to administer isotonic saline subcutaneously to both animals during and at the conclusion of cross circulation. In some instances, penicillin in saline was also administered.

11. PRESENTATION OF DATA

In all, 26 successful cross-circulation experiments were performed. Of these, all the normal animals except one were followed hematologically for a period of approximately 28 days after return to their own circulation. Cat 153 had to be sacrificed about 2 weeks after cross circulation because of a wound infection. With one exception, each cross-circulated team consisted of one radiated and one nonradiated member. The experiment involving normal cat 36, which was cross-connected in series with two radiated animals simultaneously, constituted this exception. In such a circuit, blood from the normal animal passed to one radiated animal, which was in turn connected to a second radiated animal, which likewise in turn supplied blood to the normal cat.

The data pertinent to these experiments are especially suited for presentation in graphic and tabular form, and this has been the method of presentation selected.

Figure 5.3 shows in graphic form the duration of cross circulation and the time interval (after radiation of one member of the team) that elapsed before cross circulation was established. It can readily be seen that in the majority of experiments the duration of cross circulation was in the neighborhood of 8 hr. A few animals were cross-circulated as long as 10 hr, and one for as little as 2 hr and 7 min. The animals in the group in which radiation of one partner took place at the time that the cross circulation was functioning were connected for approximately 3 to 5 hr. More experiments were performed during or shortly after the radiation of one member because it was felt that logically the most profound effects on the normal partner could be expected under these circumstances. Nevertheless, all the time intervals after radiation up to 82 hr were covered.

Tables 5.3 and 5.4 present detailed hematological data from each experiment and indicate the total leucocyte and absolute lymphocyte counts of each normal animal before and at varying intervals after cross circulation with a radiated partner. The intervals were selected arbitrarily to conserve space without sacrificing a comprehensive and representative presentation of the data. In addition, in the experiments in which one animal was shielded and the other was radiated during the period of cross circulation, the amount of radiation recorded by the ionization chamber under the shield has been indicated. In every experiment of this type a small amount of scatter radiation escaped the shielding.

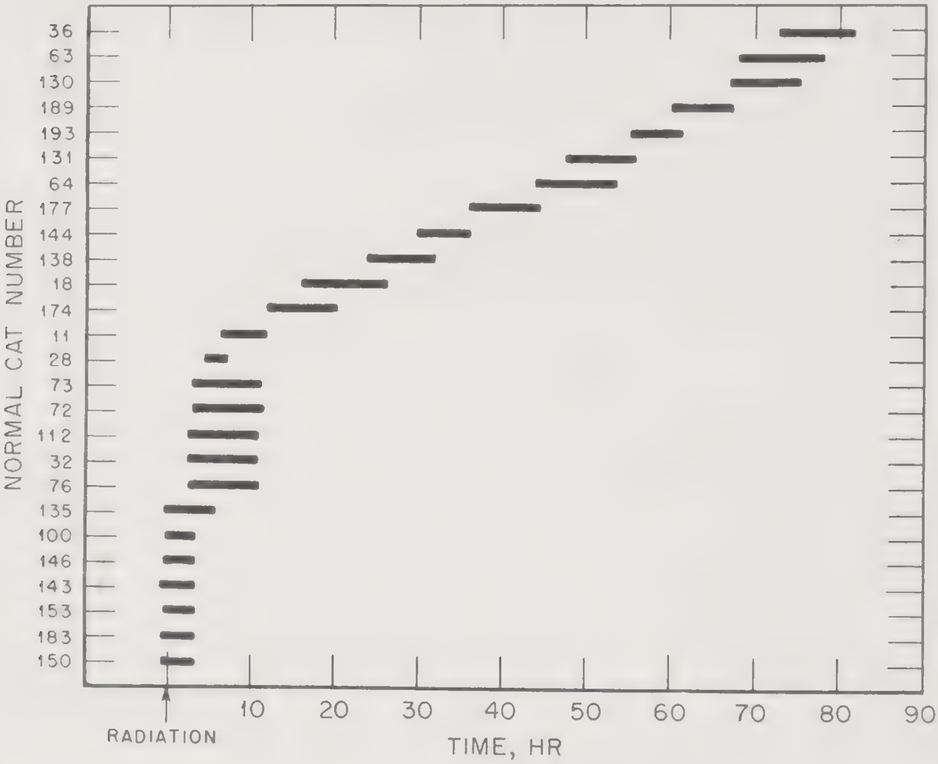


Fig. 5.3—Duration of one cross circulation. The time interval (after irradiation of one member) that elapsed before cross circulation was established is indicated.

Tables 5.5 and 5.6 give the means and standard deviations for total lymphocyte and leucocyte counts, respectively, of the normal animals

Table 5.3 — Total Leucocyte Count of 26 Normal Cats Following Cross Circulation with Radiated Animals

				Leucocytes/cu mm at indicated time after cross circulation									
Cat No.	Cross-circulation duration, hr	Radiation, r	Count prior to cross circulation, leucocytes/cu mm	First count after	1 day	2 days	3 days	5 days	7 days	10 – 11 days	14 – 15 days	21 – 22 days	End of experiment
100*	3.0	33	23,000	64,000	41,000	35,200	19,100	23,100	29,000	21,900	29,900	27,300	13,500
135*	6.0	20	14,200		31,500	25,300		13,500	19,800	27,700	21,800	12,300	9,400
143*	3.8	43	21,200	18,600	22,600	21,200	15,000		22,000	14,800	13,800	17,700	34,700
146*	3.7	28	16,400	19,000	26,700	34,700	35,100		18,300	10,800	25,200	45,900	35,500
150*	3.6	35	12,100	55,000	17,100	16,200	15,000	26,000	19,300	23,000	12,800	8,200	25,400
153*	3.3	23	5,600	19,400	24,500	15,400	25,200	36,300	19,700	31,700			
183*	3.7	24	7,800	30,600	30,500	11,300	11,400		12,200	8,400	12,800	6,900	7,500
28	2.1	None	23,800	54,500	68,800	59,500	30,250	34,300	29,600	44,900	21,700	14,300	12,000
11	5.0	None	15,200	36,100	30,100	31,900	33,400	24,500	21,500	14,800	8,900	4,200	8,400
18	10.0	None	8,800	28,900	29,000	28,300	23,100		15,900	12,400	7,800	8,000	11,100
64	9.5	None	14,000	28,300	13,800	24,500	16,200	16,500	21,000	13,700	28,500	12,700	14,600
63	10.0	None	20,800	16,100	21,000	16,000	15,600	20,900	22,000	21,200	19,100	25,500	20,200
36	8.8	None	34,700	14,000	22,500	35,700		29,100	23,900	35,000	26,100	15,600	19,900
73	8.0	None	13,200	51,500	54,100	31,400	28,200	24,600	27,000	25,700	21,000	16,100	11,000
72	8.2	None	13,600	50,800	34,300	30,900	29,400	39,200	22,300	22,100	16,700	10,600	22,700
32	8.0	None	13,700	44,900	34,200	32,100	16,400		19,000	10,500	18,700	15,000	11,400
12	8.2	None	13,800		26,300		25,800	14,500	17,300	44,600	42,700	32,800	17,500
76	8.2	None	18,700		30,700	43,000	26,700	19,000	16,600	25,600	20,100	14,300	13,200
31	8.1	None	12,100		20,600	21,700	13,200	14,300	10,600	19,000	10,300	10,300	10,200
30	8.0	None	16,300		44,700	30,900		30,500	26,400	25,000	20,700	14,400	20,000
38	7.7	None	10,500		27,400		17,200	21,800		12,800	10,800	10,700	16,700
44	6.3	None	11,900		14,300		13,100	23,600	20,300	11,400	9,300	13,700	20,500
74	8.0	None	10,900	27,000	29,000		13,400	8,500	11,300	10,100	6,300	5,700	12,600
177	8.2	None	5,900		11,200		14,700	18,200	11,700	15,100	17,200	15,500	15,200
189	7.0	None	6,000	22,800	17,800	14,500	14,500	23,600	15,600	18,900	17,300	24,700	15,300
193	5.8	None	10,200		15,500	25,900	12,000	15,000	14,800	10,100	13,800	17,700	12,400

* Shielded animals.

Table 5.4—Absolute Lymphocyte Count of 26 Normal Cats Following Cross Circulation with Radiated Animals

Cat No.	Cross-circulation duration, hr	Radiation, r	Count prior to cross circulation, lymphocytes/cu mm	Absolute lymphocytes/cu mm at indicated time after cross circulation									
				First count after	1 day	2 days	3 days	5 days	7 days	10-11 days	14-15 days	21-22 days	End of experiment
100*	3.0	33	2415	2560	1230	1232	1054	1848	3625	2080	2691	3003	1620
135*	6.0	20	3408		945	1265		1135	1683	2353	3161	3874	1692
143*	3.8	43	3180	837	2260	1696	900		2420	1628	3588	2301	3470
146*	3.7	28	4410	950	934	1561	2281		1921	864	1897	2065	1242
150*	3.6	35	1815	2750	171	243	975	780	1447	805	344	1129	386
153*	3.3	23	1708	3783	1592	2464	3006	3085	3152	4438			
183*	3.7	24	1560	1683	2135	1243	1710		854	1008	1280	1173	900
28	2.1	None	7378	5450	8256	7735	9362	6174	5032	6286	4774	6435	4200
11	5.0	None	4408	2888	3311	9889	5678	4900	3655	4440	2670	2184	3870
18	10.0	None	2904	1445	2610	849	2541		3498	2108	3354	4780	4551
64	9.5	None	2660	849	1932	3675	3240	2640	2310	4521	7980	4318	5256
63	10.0	None	5824	1449	2100	1920	3432	5852	3520	3604	4584	8415	5454
36	8.8	None	8328	1820	3150	2499		5820	3107	3500	3654	4524	2587
73	8.0	None	3564	1030	1082	7536	5358	4183	3510	1542	2310	2415	2860
72	8.2	None	3128	2032	3087	4635	4998	3920	5352	5525	5010	1708	2270
32	8.0	None	3151	2245	2394	4173	5084		4465	1470	4488	2850	3534
12	8.2	None	3312		1052		3354	1450	3114	3122	2989	2624	5425
76	8.2	None	2244		2149	1720	2136	1710	3652	2560	2010	2860	1188
31	8.1	None	3267		2884	1953	3168	1716	2120	5130	2266	2266	2856
30	8.0	None	2608		2682	2472		2440	1848	4000	2691	2592	1800
38	7.7	None	2625		822		2236	3488		896	2376	1928	1336
44	6.3	None	3332		1287		2358	2124	3248	2052	1023	959	3485
74	8.0	None	1090	0	0		1742	1105	1582	1111	630	798	1764
177	8.2	None	2478		1680		2352	2730	702	2265	3268	3255	3648
189	7.0	None	2040	0	1780	1740	2030	1888	624	1512	2768	1976	1989
193	5.8	None	3162		3410	7770	3120	2250	2220	2626	4830	4248	3720

* Shielded animals.

Table 5.5—Means and Standard Deviations for Absolute Lymphocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals

	Shielded group			Nonshielded group			Entire group		
	No.*	Mean	S.D.	No.*	Mean	S.D.	No.*	Mean	S.D.
Prior to cross circulation	7	2642	1063	19	3552	1798	26	3307	1664
First count after cross circulation	6	2093	1145	11	1746	1515	17	1868	1368
1 day after	7	1323	734	19	2403	1697	26	2112	1562
2 days after	7	1386	665	14	4183	2892	21	3251	2719
3 days after	6	1804	1159	17	3658	1928	23	3174	1924
5 days after	4	1712	1017	17	3199	1657	21	2916	1646
7 days after	7	2157	974	18	2975	1320	25	2746	1270
10–11 days after	7	1882	1279	19	3066	1579	26	2747	1573
14–15 days after	6	2160	1221	19	3351	1675	25	3065	1638
21–22 days after	6	2257	1063	19	3191	1849	25	2967	1722
End of experiment	6	1551	1056	19	3252	1346	25	2844	1463

* Number of animals on which counts are available at the time specified in column 1.

Table 5.6 — Means and Standard Deviations for Total Leucocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals

	Shielded group			Nonshielded group			Entire group		
	No.*	Mean	S.D.	No.*	Mean	S.D.	No.*	Mean	S.D.
Prior to cross circulation	7	14,328	6,461	19	14,426	6,658	26	14,400	6,476
First count after cross circulation	6	34,433	20,133	11	34,081	14,440	17	34,205	16,032
1 day after	7	27,700	7,624	19	28,700	14,484	26	28,430	12,853
2 days after	7	22,757	9,440	14	30,450	11,216	21	27,885	11,060
3 days after	6	20,133	8,714	17	20,185	7,254	23	20,171	7,452
5 days after	4	24,725	9,385	17	22,241	7,860	21	22,714	7,977
7 days after	7	20,042	4,988	18	19,266	5,510	25	19,484	5,278
10–11 days after	7	19,757	8,704	19	20,678	10,798	26	20,430	10,115
14–15 days after	6	19,383	7,323	19	17,736	8,665	25	18,132	8,246
21–22 days after	6	19,716	14,826	19	14,831	6,872	25	16,004	9,260
End of experiment	6	21,000	12,572	19	14,994	4,153	25	16,436	7,261

*Number of animals on which counts are available at the time specified in column 1.

for the same time intervals before and after cross circulation as given for the individual animals in Tables 5.3 and 5.4. The figures are divided into three groups: those applying to the shielded group of normals, which were cross-circulated at the time of their partners' radiation; those applying to the nonshielded group, which were cross-circulated with their previously radiated partners; and those applying compositely to the group as a whole. It will be noted that the number of animals for which data are presented varies somewhat on different days. This is due to the fact that counts were made only 6 days a week so that at any specified interval after cross circulation it is possible that counts are not available for every member of the group.

Figure 5.4 indicates graphically a composite picture of the average leucocyte and lymphocyte counts of the entire group during the period of follow-up. In addition, the same data on the seven shielded animals are graphed separately, both because these animals unavoidably received small amounts of radiation and because this type of experiment was considered the most critical of the group.

12. RESULTS

Analysis of the data indicates few or no detectable hematological abnormalities in the normal cat at any time after its cross circulation with a radiated animal. The average leucocyte count for the entire normal group and for the critical group, in which cross circulation was established at the time of their partners' radiation, remains throughout the entire approximately 28-day period of follow-up actually somewhat higher than the pre-cross-circulation control level. In not even a single animal did evidence of significant leucopenia develop at any time during the experiment, despite the fact that animals received for a period of several hours the full volume output of the carotid artery from a radiated partner. As would be expected, well-marked leucocytosis was present in many instances for a few days after the operative procedure. Its duration varied rather widely from animal to animal. Of some interest is the striking degree of leucocytosis that developed at the time of cross circulation in certain animals whose partners had received their radiation at the time of or shortly before cross circulation. Whether these more striking instances of leucocytosis are due to the passage of leucocytotic metabolites that resulted from tissue breakdown as a result of radiation cannot be determined with certainty. The leucocyte counts in question were markedly in excess of those developed in a normal control group, however.

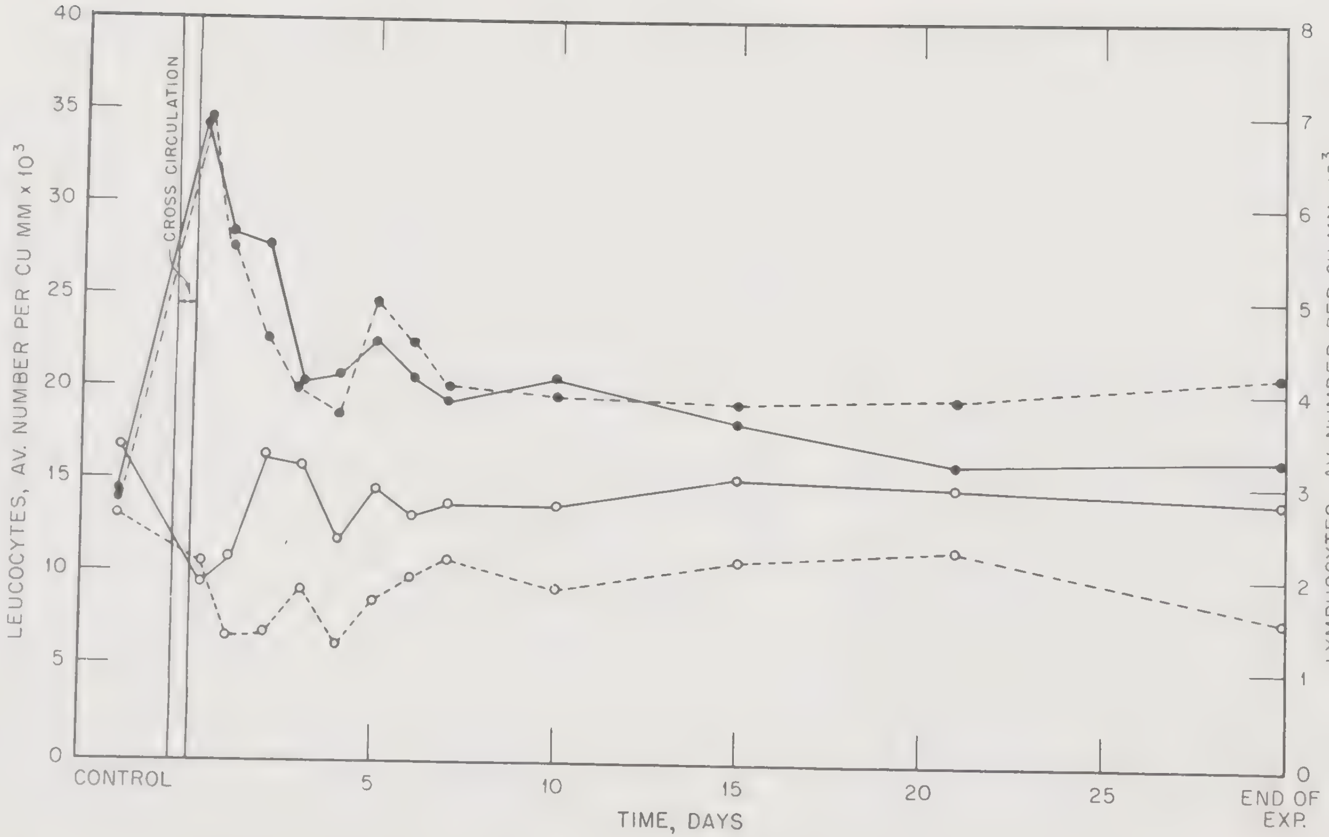


Fig. 5.4—Average leucocyte and lymphocyte counts of the entire group of animals for the period of the experiment. The average leucocyte and lymphocyte counts for the critical shielded group are presented separately. •——•, average number of leucocytes for the entire group. •---•, average number of leucocytes for the shielded group (seven animals). ○——○, average number of lymphocytes for the entire group. ○---○, average number of lymphocytes for the shielded group.

Individual lymphocyte values found in normal animals after cross circulation show a moderate amount of variation. As would be expected during the period of marked leucocytosis during the few days immediately following cross circulation, a drop in the absolute lymphocyte count was noted. Lymphopenia is a well-known accompaniment of neutrophilic leucocytosis and in this instance was presumably accentuated by the fact that some animals had been cross-circulated with partners who had no circulating lymphocytes at all. To be sure, the average lymphocyte level for the entire group remained throughout slightly lower than that found before cross circulation, and this trend was slightly more apparent in the critical shielded groups as a whole. In view of the physiological variations in lymphocyte counts and the persistence of some increase in the average total leucocyte counts throughout the experiment and, particularly, in view of the fact that the reduction in lymphocyte count was relatively slight and not at all of the order of magnitude associated with direct radiation, there is grave question that any significance can be attached at all to this finding. At no time did the average percentage of lymphocytes fall below 4.9 per cent of the leucocytes present even in the shielded group, and this value was obtained only for the first day after radiation. In all other instances it was appreciably higher than this.

13. DISCUSSION

It is apparent that, under the conditions of these experiments, no specific indirect effect of radiation has been demonstrated. It is also felt that, if significant indirect effects peculiar to radiation are dependent on the circulation, they should have been readily demonstrated by the experimental setup employed. However, it cannot be denied that if it had been possible to maintain cross circulation for much longer periods, e.g., 48 or 72 hr instead of 8 to 10 hr, indirect effects too slight for demonstration during the shorter period might have become manifest. The possibility of an indirect radiation effect mediated solely by the lymphatics was also considered but was discarded as highly unlikely. Any lymphatic disseminated agent would in all probability soon reach the venous and arterial tree and would be expected to manifest itself in the blood. Furthermore, if in some devious manner this agent should be removed from lymph before reaching the blood, indirect effects of radiation would exist purely in the regional lymphatic drainage area of that portion of the body directly radiated. The existence of such a purely localized regional indirect radiation effect is supported by no evidence and, in fact, is contrary to whatever evidence there is that is suggestive of such indirect effects.

It is also apparent that there is no satisfactory evidence for a characteristic indirect radiation effect presented in the medical litarature. Such evidence as is presented is of dubious character and is refuted, for the most part, by contrary results obtained in similar types of experiments by other investigators. There has been no more than a desultory attempt on the part of investigators of indirect radiation effects to differentiate body reactions common to a variety of damaging stimuli from those which are specific to radiation. This is a fundamental differentiation, particularly in view of Selye's now well-supported observations that an identical nonspecific systemic reaction (at times severe enough to result in death) occurs as a response to many unrelated injurious stimuli. Still, it is undoubted that leucopenia develops in a certain number of individuals treated with X rays, even when only an insignificant amount of marrow has been directly exposed to radiation.

Kornblum and associates[52] in an extensive series of clinical observations concluded that therapeutic radiation tended to lower the leucocyte count. However, in approximately one-half the patients studied there was no decrease in the number of neutrophils, and in the great majority of the remaining cases depression was of slight degree. In only 7 cases out of 120 were the neutrophils reduced below 2000/cu mm. The effect of therapeutic radiation on the lymphocytes was more striking, a definite decrease in their numbers being the rule. In most cases the drop in count was relatively slight, but in some instances it was very pronounced. In this study there was no apparent relation between the effects on the leucocytes and the part of the body treated, the amount of radiation, and the time during which the patient was irradiated. However, the effect appeared less marked, the smaller the size of the areas treated. It is also highly likely that involution of and histological changes in normal and abnormal lymphoid tissue remote from the site of direct radiation as observed by many roentgenologists and research workers is real and not apparent. It would seem wise to examine these effects more closely to see if it is necessary to assume that they are peculiar to radiation and if they can accurately be described as radiation effects per se.

Leucopenia, although not unusual, is a highly inconstant feature of radiation therapy. There is no close relation between its occurrence and the dosage of radiation employed, and it is impossible to predict beforehand which individuals will develop it and which will not. If it is an attribute of radiation per se, it is a highly capricious one.

Moreover, the granulocytopenia of the type under discussion bears a rather superficial resemblance to the well-established direct effects of radiating a large amount of bone marrow. In the former case, the leucocyte count frequently falls to the neighborhood of 3000/cu mm and then remains at approximately that level even with continued therapy; in the latter case, agranulocytosis is often extreme, and further generalized radiation invariably contributes to its perpetuation. In the former instance, marrow specimens are of normal cellularity or even hyperplastic, and the myelopoietic elements are normal except for some reduction in the percentage of adult granulocytes; in the latter instance, hypocellularity and aplasia are the rule. In order to correlate the available evidence, it would therefore be necessary to postulate that the direct and indirect effects are distinct except for some superficial resemblances and that in the case of the indirect effect it is inconstant, poorly related to dosage, and totally unpredictable. It seems more reasonable to assume that, in an organism capable of manifesting peripheral leucopenia in response to a wide number of injurious agents, products of cellular breakdown and abnormal metabolites resulting from the radiation injury result in the development of leucopenia in certain susceptible individuals. It does not seem necessary on theoretical grounds to assume that the action of radiation did more than produce the initial injury or that it occurred at other than the direct site of exposure.

Substantial involution of lymphoid tissue not in itself directly injured has been frequently observed as a nonspecific response to a wide variety of injurious agents. Bardeen,[53] in studying visceral changes occurring in patients dying of superficial burns, noted striking histological changes of a degenerative nature in lymphoid tissues and commented on the similarity of these changes to those found experimentally after injection of ricin or diphtheria toxin. These widespread alterations in all the lymphoid organs of the body following burns have been amply confirmed by other investigators.[38,54,55] Similar changes and transient leucopenia and lymphopenia have been observed in mice subjected to dry heat in nonfatal exposures.[56,57] Selye,[50,58,59] in what he has termed the "alarm reaction," has described striking involutionary and degenerative changes in all the lymphatic organs as a nonspecific response to a variety of insults (cold, heat, surgical shock, and drugs). Nonspecific damage apparently may result in atrophy of the spleen beginning in the center of the Malpighian corpuscles, marked loss of weight of thymus and lymph nodes, and, at times, even complete disappearance of germinal centers in the lymph nodes. Zecker[60] noted similar changes after subcutaneous tissues were injured by injection of formalin. It is interesting that this involution of lymphoid structures does not occur in the adrenalectomized animal, particularly in the light of recent investigations which suggest that lymphoid structures and the lymphocytes may be at least partly under adrenal-cortical control.[61-68] In view of the above strongly supported observations, it is quite possible that X radiation is merely one of many injurious agents capable of producing generalized involution of lymphoid tissue as a nonspecific response to local injury. This, of course, is not to be confused with local involution and degeneration of lymphoid tissue directly exposed to radiation.

The question of whether a specific indirect effect of radiation exists cannot be regarded as settled. However, it is felt that the observations presented weigh appreciably against the possibility, and analysis of the evidence and arguments for the existence of such effects indicates little of a valid and concrete nature to support it.

14. SUMMARY

The general aim of the investigations reported here has been to obtain evidence for or against indirect radiation effects. To this end, 26 successful cross-circulation experiments (carotid-to-carotid anastomoses) have been performed between normal cats and radiated cats. Cross circulation was established in most instances at some specified time interval after the radiation of one partner. All intervals up to 82 hr after radiation of one partner were covered.

In seven experiments, cross circulation was established, and then one animal was radiated while the other was shielded. These were considered the most critical experiments of the group.

Detailed data on leucocyte and lymphocyte counts in the normal animal obtained during an approximately 28-day period of follow-up are presented.

These data are not considered to support the thesis of indirect effects peculiar to radiation. A trend toward slightly lowered absolute lymphocyte counts in normal animals after cross circulation was not considered significant, and in no instance did leucopenia develop in the normal animal.

The literature is reviewed and discussed.

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Chapter 6*

FINGER RIDGE CHANGES IN MONKEYS FOLLOWING X RADIATION

By Roger A. Harvey

Surveys of professional and industrial groups of human beings possibly exposed to short-wavelength radiations have shown a relatively high percentage of abnormalities of finger ridge detail when compared with other groups not similarly exposed. However, very little information was obtained in regard to the dose necessary to produce these changes or in regard to the pathology underlying the gross changes observed. The monkey was chosen for these additional studies because of the similarity of the ridges of its palms and fingers to human ridges.

1. METHOD

Ridge details of the hands of the monkeys were recorded in the same impression compound that was used in earlier surveys of man (see Fig. 6.1). The monkeys' hands were thoroughly but gently washed in soap and water, dried, and then wiped with an oil-moistened cloth. The hands were placed palmar surface down on the softened compound, and sufficient pressure was applied to the dorsum of the hands and fingers to force the ridge-bearing area from 1 to 3 mm into the compound. No attempt was made to make impressions of single fingers or to roll the fingers from one side to the other in making the record. The hands were small enough to permit recording of detail from all the fingers and distal half of the palm at one time on a single block of compound. Control impressions were made from each monkey before X-ray exposure. Subsequent impressions were made every 2 weeks after completion of the acute and chronic exposures.

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*Based on Report MDDC-215 (II-188-5940).

For treatment, the monkeys were restrained face up on animal boards with forearms extended above the head. The hands were held palmar surface toward the X-ray tube. Lead was placed over the forearms, palms, and thumbs during exposure so that ridge detail in the palms and thumbs could be used for further control purposes (Fig. 6.2). The entire length of all the fingers was irradiated.

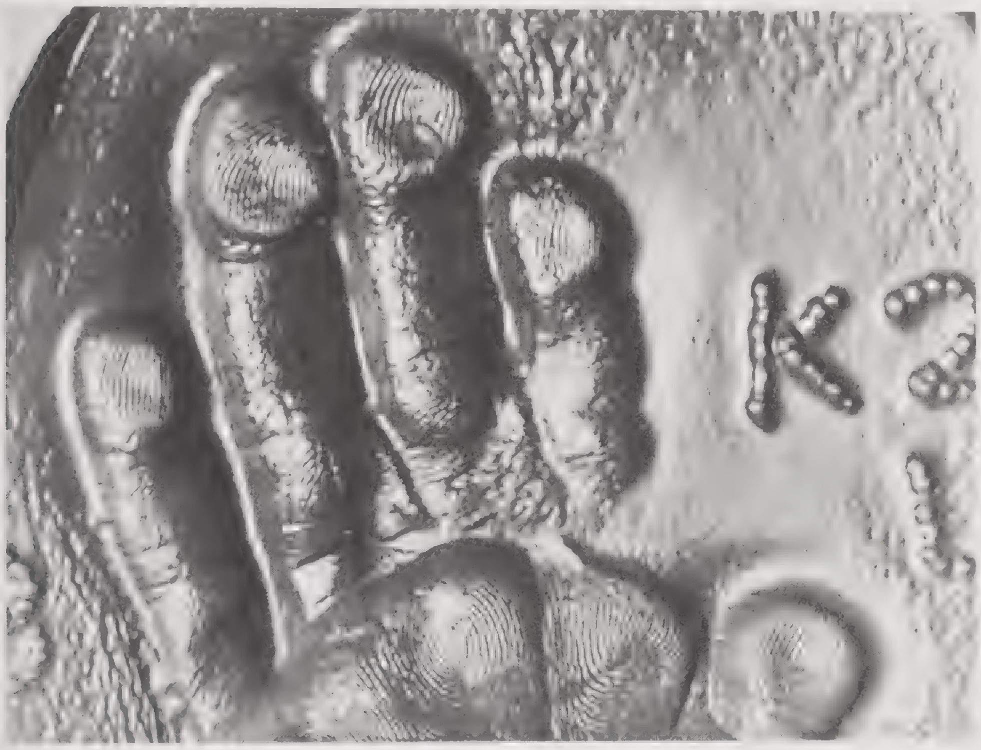

Fig. 6.1 — Pretreatment record showing the ridge detail present in a monkey's hand as recorded in the impression compound.

The monkeys were kept until death from natural causes. Sections were taken from the volar surface of each finger at autopsy for microscopic examination.

The exposure factors for the experiments are given in Table 6.1. The changes in factors between acute and chronic experiments were necessary because the high intensity of output in the acute experiment made accurate timing of the very small doses for the chronic experiments impossible with available equipment. The change in factors between acute experiments was necessitated by complete change of available equipment.

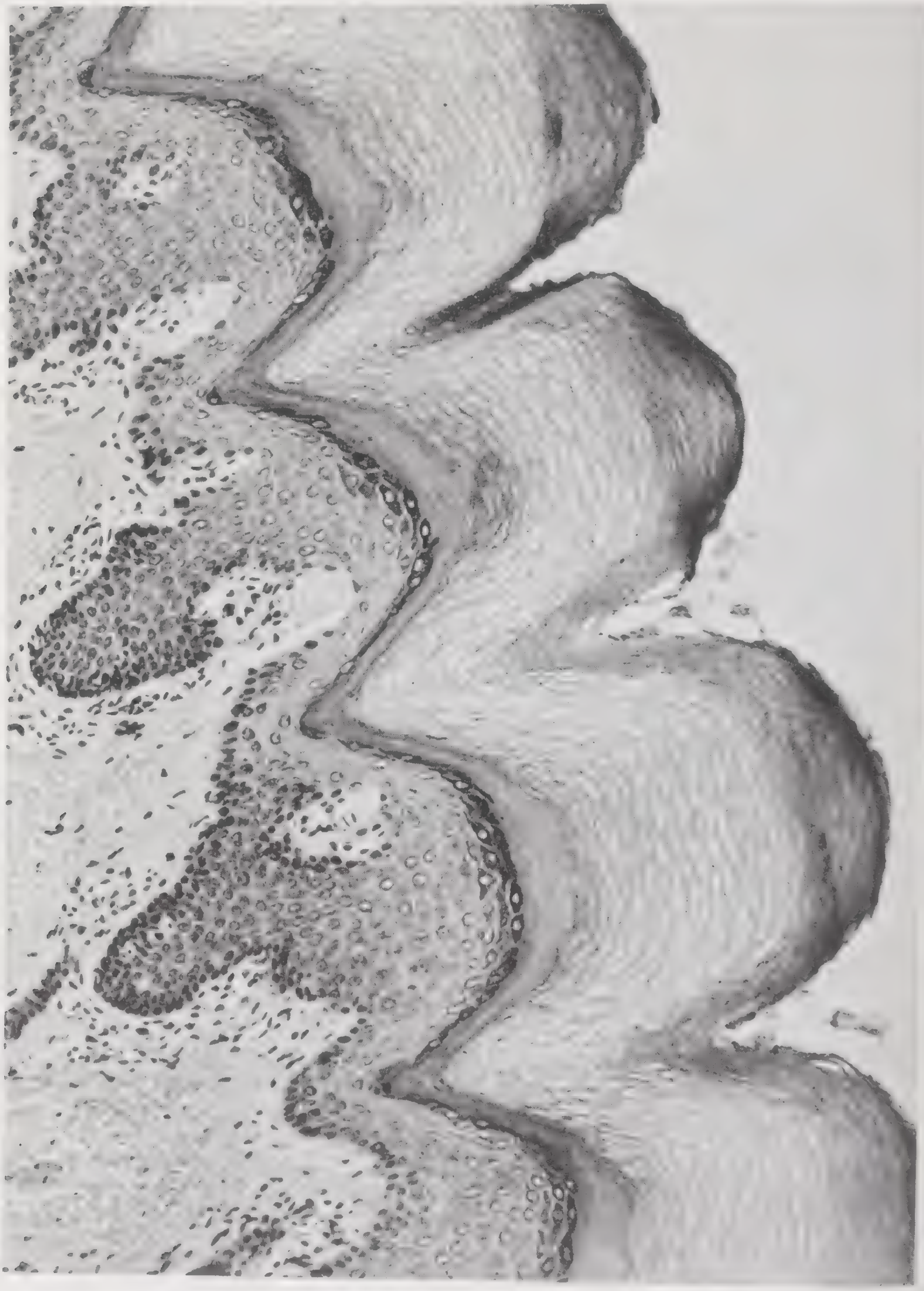

Fig. 6.2—Section of tissue removed from the fingerprint area of the thumb (control area). Note the uniformity of contour of the upper surface and the clarity of layers and component cells. 170 ×

Table 6.1 — Exposure Factors

Monkey No.	Dose to right fingers, r	Dose to left fingers, r
	A. Acute Experiment*	
1	400	600
2	500	700
3	800	1000
4	900	1100
5	1200	1400
6	1300	1500
7	1600	1800
8	1700	1900
9	2000	2200
10	2100	2300
11	2400	2600
12	2500	2700
13	Control	Control
	B. Acute Experiment*	
14	1000	1400
15	1400	1800
16	1800	2200
17	2200	2600
18	2600	3000
19	3000	3400
20	3400	3800
21	3800	4200
22	4200	4600
23	4600	5000
	C. Chronic Experiment*	
24	10 × 50	20 × 50
25	20 × 50	30 × 50
26	30 × 50	40 × 50
27	40 × 50	50 × 50
28	50 × 50	60 × 50
29	60 × 50	70 × 50
30	Control	Control

*The conditions for the experiments were as follows: A: 80 kv, 10 ma; target-skin distance (TSD), 7 in.; no added filter; half-value layer (HVL), 0.9 mm Al; and dose rate, 440 r/min in air. B: 80 kv, 10 ma; TSD, 11 in.; no added filter; HVL, 0.9 mm Al; and dose rate, 432 r/min in air. C: 80 kv, 1 ma; TSD, 20 in.; no added filter; HVL, 0.9 mm Al; dose rate, 60 r/min in air 6 days a week for 50 treatments.

2. RESULTS

The monkeys in all colonies showed a discouraging incidence of tuberculosis, and most of them died before complete effects of irradiation could be ascertained. The main reasons for undertaking the second acute experiment were to overlap the critical level of the first experiment and to carry the doses high enough so that cyclic changes might have a chance to become more apparent before animals in this second group died of intercurrent infection.

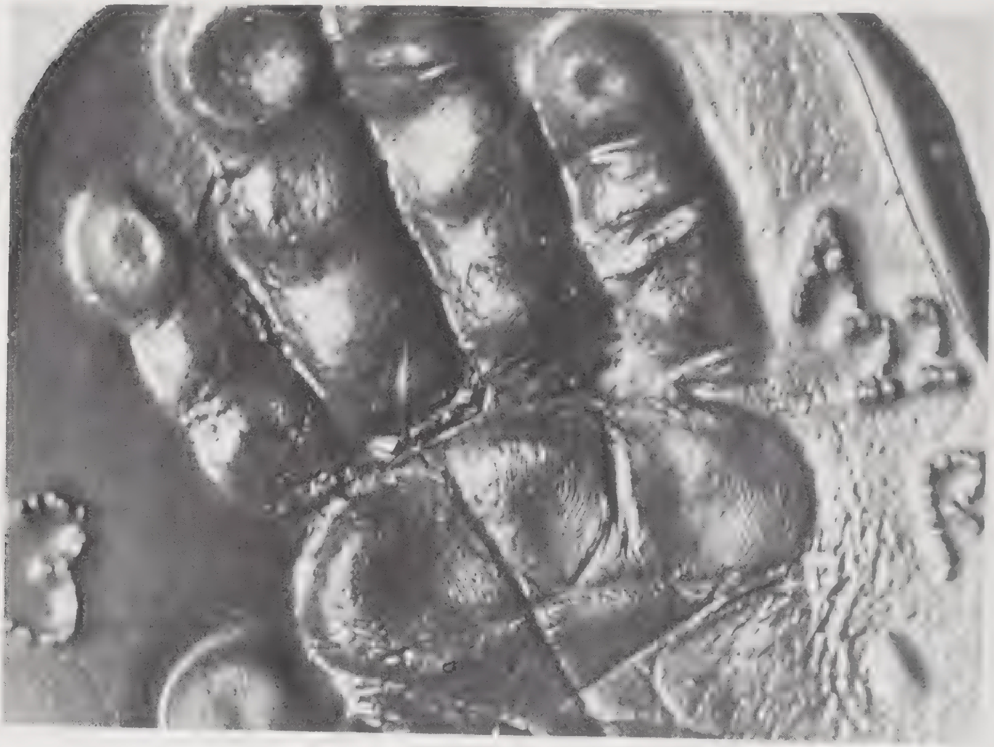

Fig. 6.3—Typical findings 3 weeks after an acute exposure to 4200 r in air. This is the stage of edema and desquamation.

The first effect of acute doses of X rays was edema, which was recognized 2 weeks after exposure of 1200 to about 2300 r and about 8 to 10 days after doses of 2400 to 4600 r. The edema was so marked in the higher dose range that no ridge detail could be detected 3 weeks after exposure (see Fig. 6.3). Loss of pigment became prominent about 2 weeks after treatment with doses greater than 2600 r. Desquamation was conspicuous 1 month after treatment with 2600 r or more. Patchy areas of ridge regeneration appeared as soon as the edema subsided in the lower dose range, and regrowth proceeded steadily (Figs. 6.4 and 6.5). With larger doses, regeneration was delayed or entirely inhibited. When it did occur, the rate of regrowth was much slower than with smaller doses, and there was a tendency for secondary weakening of detail when initial regeneration appeared after large doses. There was definite deep atrophy of the fingers with doses above 3000 r.

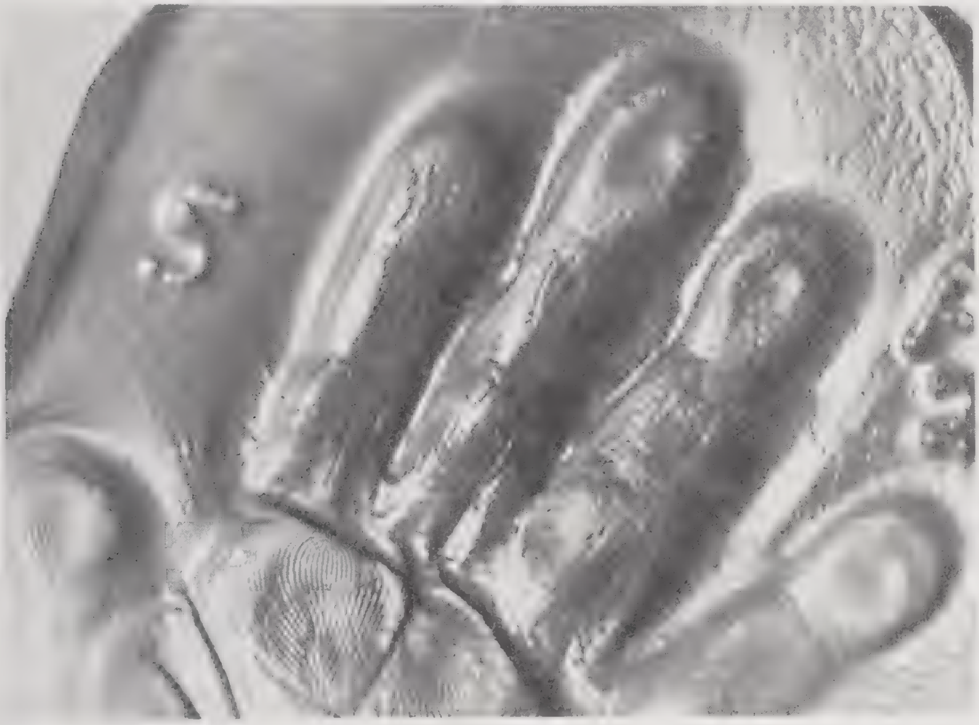

Fig. 6.4—Impression of the hand shown in Fig. 6.3, 5½ months after exposure. Ridges are barely perceptible, and longitudinal wrinkling of the skin, which is indicative of deeper atrophy, is beginning.

In the chronic-dose experiment, 10 r/day for 50 doses caused no detectable change in ridge detail. Doses of 20 to 30 r/day caused very minimal weakening of ridges without any evidence of return to normal 6 and 7 months after treatment (Figs. 6.6 and 6.7). Doses of 40 to 70 r/day caused more severe weakening of ridges, partial regeneration, and secondary weakening.

The results of experiments A, B, and C (Table 6.1) are shown graphically in Figs. 6.8 to 6.10, respectively. All measurements of ridge height are estimates from the impression compound and are

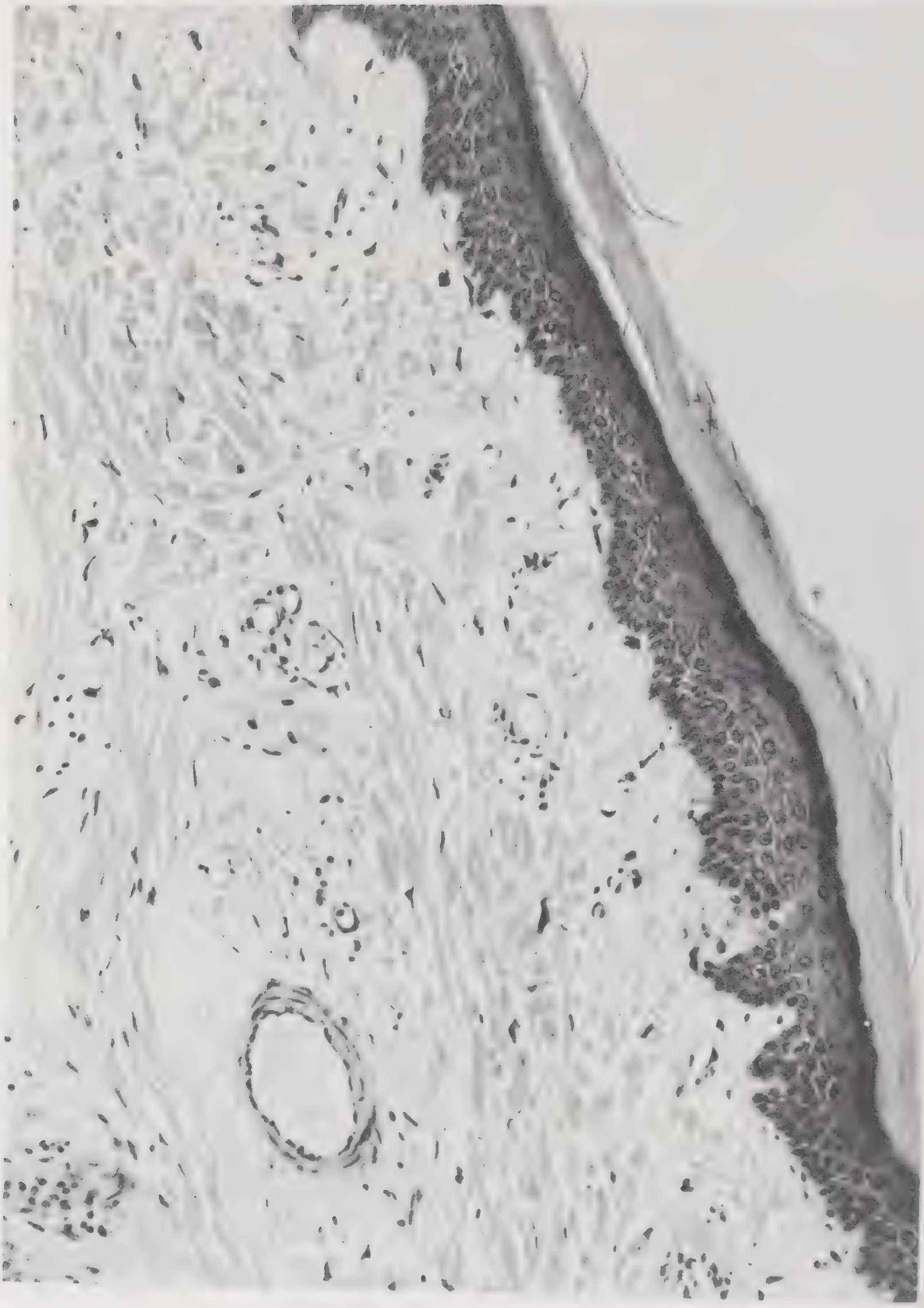

Fig. 6.5—Section of tissue removed from the fingerprint area of the index finger of the hand shown in Fig. 6.4, 5½ months after an acute exposure to 4200 r in air. Note the loss of all ridge contour, the marked thinning and keratinization of the stratum corneum, the indistinct stratum granulosum, the markedly flattened dermal papillary zone with loss of almost all ridges and pigment, and the reticular-appearing zone underlying the papillary layer. Blood vessels are prominent but slightly thickened. 170 ×

plotted in relation to height during the control period. The initial left-hand point of each line indicates the initial pretreatment height of ridges, and the base line represents the zero point of ridge detail.

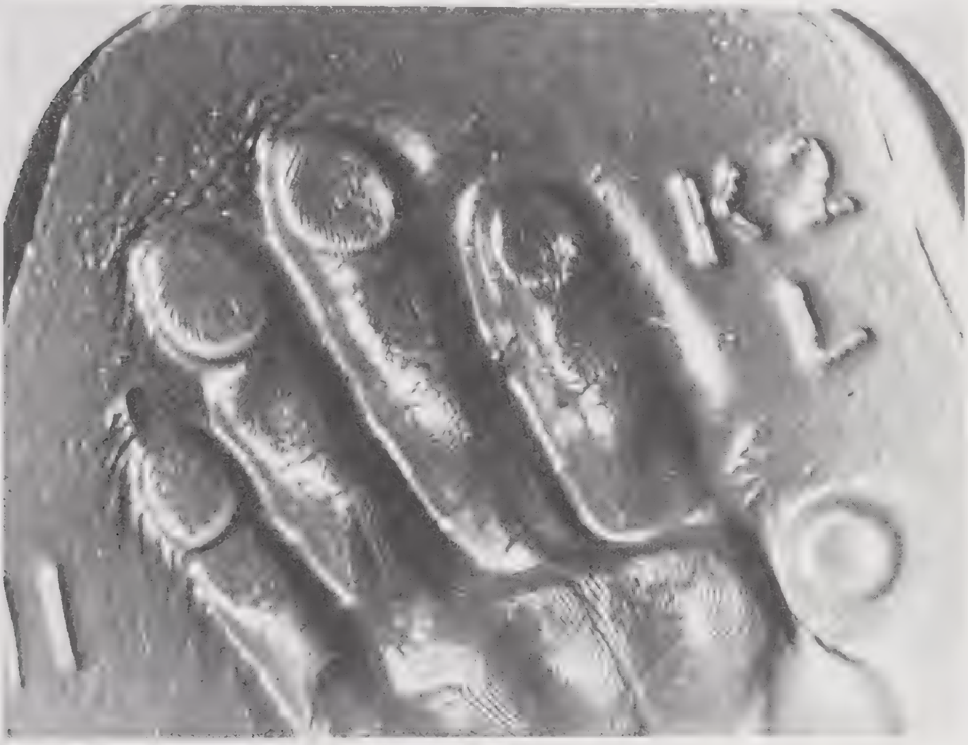

Fig. 6.6—Impression of the hand shown in Fig. 6.1, 6 months after chronic exposure to 30 r/day for 50 exposures or a total of 1500 r in air. Note the degree of thinning of ridge detail.

3. DISCUSSION

Although the results of these experiments are similar to those observed in experiments on human beings, the dose of X rays necessary to produce minimal changes in monkeys and man may not be very close. The skin on the volar surface of a monkey's finger is much tougher than that of man. This was dramatically brought out in the case of all the monkeys receiving 1800 r or more in an acute dose. All these monkeys showed such long fingernails 4 to 8 weeks after treatment that they were impeded in picking up food. Under normal conditions a monkey will keep its nails short from scratching and in so doing will subject the ridges of the fingerprint to considerable wear although detail remains superb. The nails tended to become

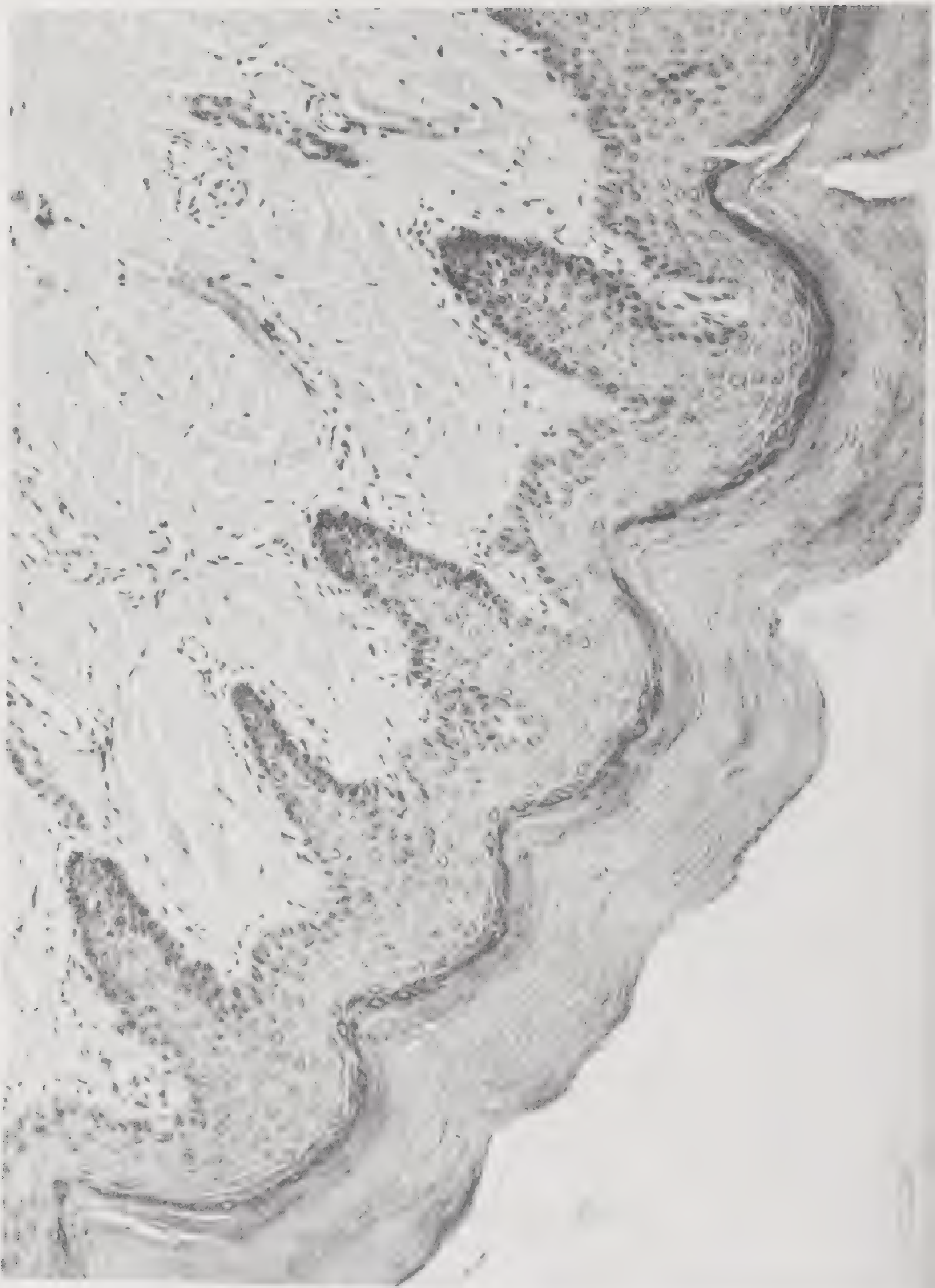

Fig. 6.7—Section of tissue removed from the fingerprint area of the index finger of the hand shown in Fig. 6.6, 6 months after chronic exposure to 30 r/day for a total dose of 1500 r. Note the loss of uniformity of contour of the upper surface, the thinning and keratinization of the stratum corneum, the less sharply outlined cells of the stratum granulosum, the narrower dermal papillae with less distinct and more pigmented marginal cells, and the sclerosis of the papillary zone. 170 ×

normal in length 8 to 12 weeks after exposure to doses up to about 3000 r, but, from that level on, atrophy became apparent. The nails curled, dried, narrowed, and grew much slower.

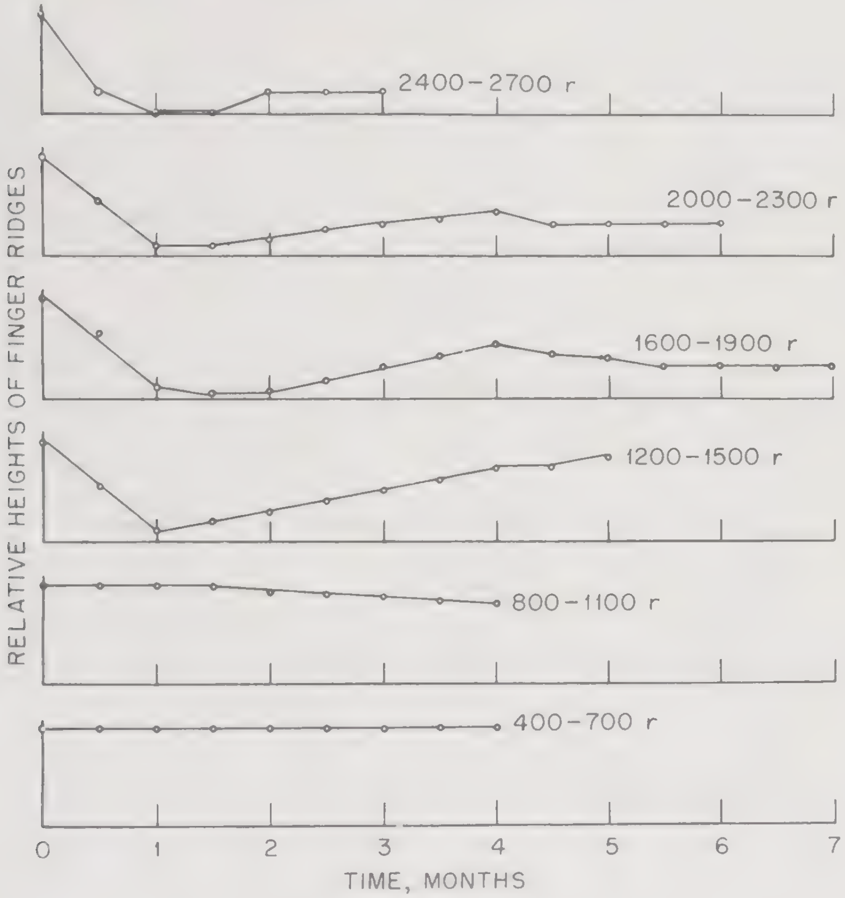

Fig. 6.8—Finger ridge response in monkeys in acute experiment A.

It is surprising that an acute dose in the 800- to 1100-r range and a total chronic dose of 1000 r will produce detectable changes in monkey finger ridge details. Although the time of onset is much earlier and the degree of change more severe with the acute dose than with the chronic dose, from clinical experience it was expected that a much larger chronic than acute dose would be required to produce detectable changes.

The tendency for regeneration to be followed by secondary weakening of ridges in some cases may account for some of the phenomena observed in industrial groups where severity of damage varied in series of impressions from the same individuals.

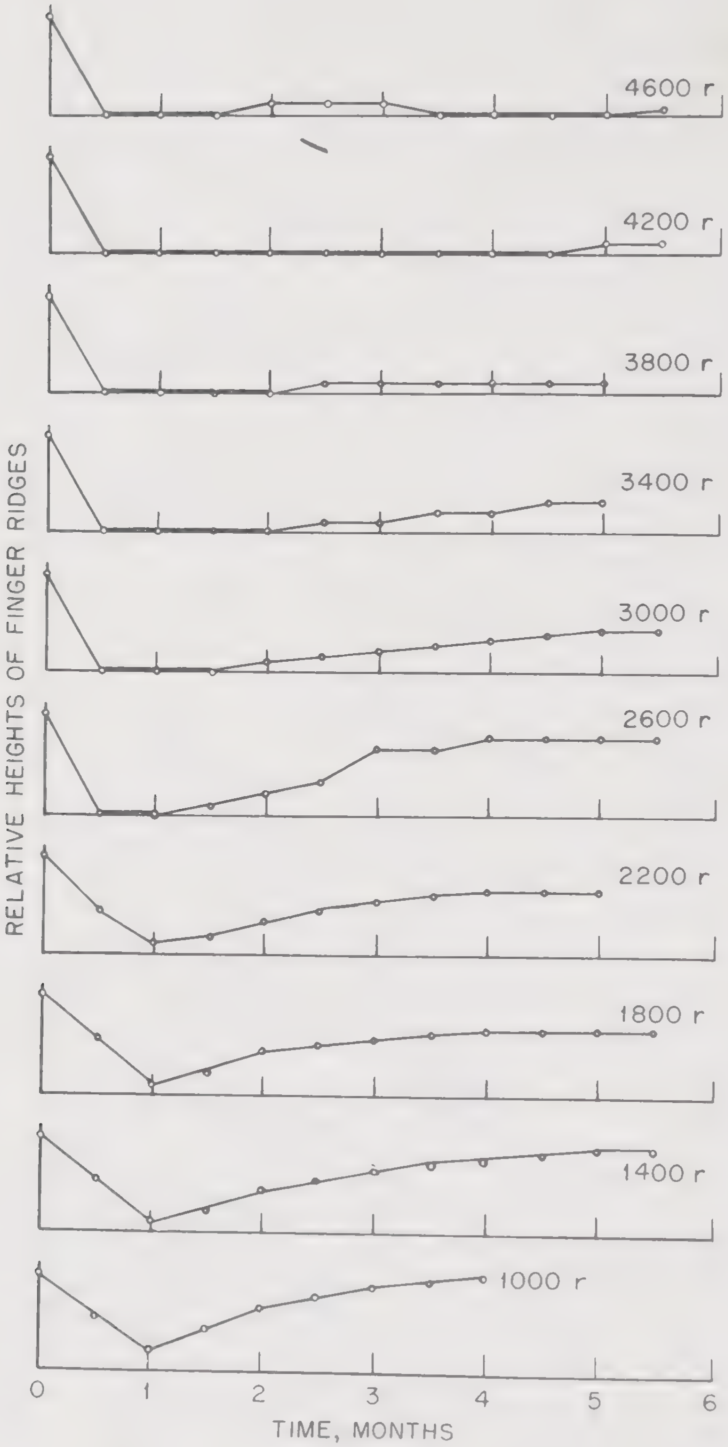

Fig. 6.9—Finger ridge response in monkeys in acute experiment B.

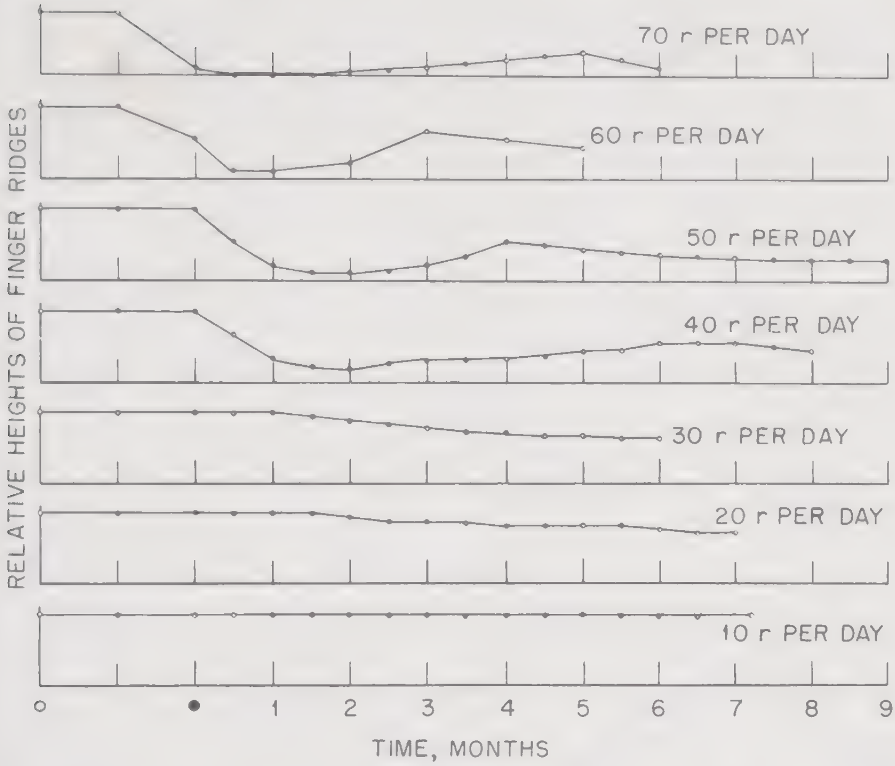

Fig. 6.10—Finger ridge response in monkeys in chronic experiment C. ○, before treatment. ● , day of last treatment.

4. CONCLUSIONS

Within the time limits of these experiments the following conclusions were made:

1. Acute X-ray exposures up to 800 r cause no detectable gross changes in finger ridge details of monkeys. Acute doses of 800 to 1100 r result in temporary atrophy of ridges. Acute doses above this level cause more severe atrophy, slower regeneration, less complete regeneration, and some tendency to secondary weakening of detail.
2. Chronic X-ray exposures of 10 r/day for 50 treatments cause no change in ridge detail. Chronic exposures of 20 r or more per exposure for 50 exposures cause detectable weakening, which, like that following acute exposures, is more severe with increasing dosage and shows a slower tendency to regenerate, less complete regeneration, and more secondary weakening.

3. Similar total doses of acute and chronic X-ray exposure yield more severe initial changes in ridge details with acute than with chronic exposure, less complete regeneration with chronic than with acute exposure, and slightly more secondary weakening of ridge detail with chronic than with acute exposure.

Chapter 7*

EXPERIMENTS ON THE VALIDITY OF THE LINEAR RELATION OF MUTATION FREQUENCY TO X-RAY DOSE IN DROSOPHILA MELANOGASTER

By Warren P. Spencer and Curt Stern

1. INTRODUCTION

Since the discovery by Muller[1] and Stadler[2] that X rays induce mutations in living organisms, a large amount of data has been accumulated by many workers dealing with the relation of mutation frequency to dosage intensity. X rays of various wavelengths, radiations of radium, neutrons, and ultraviolet light have all been used as causative agents. In this chapter the voluminous literature that has been written in this field is not reviewed; the reader may refer to the general reviews of Schultz,[3] Stubbe,[4] and Timoféeff-Ressovsky.[5]

On the basis of the accumulated data on Drosophila melanogaster, on which most experiments have been conducted, and with allowance for variables not easily controlled from experiment to experiment, radiation geneticists are generally agreed that the relation of dose to mutation frequency seems to be linear. Furthermore, experiments on fractionation of dosage and variation in time-intensity relations would seem to indicate that this linear relation should hold at very low dosages. Although the extrapolation of the curve into the region of low dosages is a reasonable hypothesis, the experimental investigation of this part of the curve is of interest. From a theoretical point of view the validity of the extrapolation needs to be checked by observation. This part of the curve is of special significance from the practical aspect of the effect of low-dosage radiation on man.

The lowest dosages thus far used have been 385 r for X rays by Oliver[6] and 400 r for radium by Raychaudhuri.[7,8] The accumulation of sufficient data to be statistically significant for lower dosages than the ones reported is a considerable task. In this chapter, data on controls and 25, 50, 500, 1000, 2000, 3000, and 4000 r will be reported. On the basis of the results of a study of 200,801 test cultures for lethals in the X chromosome of the fruit fly, Drosophila melanogaster, of which 73,901 were controls, 51,907 from flies irradiated at 25 r, 31,560 at 50 r, 23,195 at 150 r, and smaller numbers at the higher dosages used, it is necessary to conclude that there is no tolerance dose of radiation by means of X rays below which mutation does not occur. These data indicate that a dosage of about 50 r actually doubles the natural lethal mutation rate in flies. Less extensive data on visible mutations show the same relation.

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*Based on Report MDDC-765 (II-188-6524), which has appeared in the published literature [Genetics, 33: 43-74 (1948)].

If these findings can be shown to have transfer value to the effect of radiation on human beings through corroboration by experiments on the mouse, a mammal, then it becomes clear that radiation in dosages which may be tolerated by the body of man may have unfavorable effects on the human germ plasm. In terms of society and human beings the risks can only be stated when a statistic on the proportion of individuals of reproductive age exposed to low-dosage radiation is introduced. For the individual exposed and his descendants the risk is obviously much greater. These facts should be carefully considered in any proposed use of atomic energy on a large scale.

The collection of the data reported in this chapter occupied the full time of three workers in the laboratory from October 1943 to July 1945. In addition to the main problem of induced mutation frequency at low dosages, some data are presented on the ratio of pure lethals to visibles, to semilethals with visible effects, and to semilethals without visible effects; the occurrence of lethal clusters in controls and such clusters at low dosages; the association of lethals with crossover modifiers at different dosages; and the distribution of lethals and visibles on the genetic map of the X chromosome in Drosophila melanogaster. For the benefit of future workers in this field, a section is included on problems involved in large-scale experiments of this type (Sec. 8).

2. DESCRIPTION OF TECHNICAL METHODS

2.1 Genetic Procedure. Through the courtesy of H. J. Muller, of Indiana University, whose cooperation and advice have been invaluable, a tool stock was obtained for use in making tests of lethals and visibles in the X chromosome of control and irradiated flies. For convenience this stock has been designated "Muller 5," and this name will be used throughout this chapter. The complete formula for the stock is sc^{SI} B In-S w^{a} sc^{8}. Muller, in a written communication, explains the genetic content of the stock as follows:

> The expression $sc^{SI}\ldots sc^{8}$ refers to a chromosome whose left end is derived from the $scute^{SI}$ inverted X chromosome whose right end is from a $scute^{8}$ chromosome. Such a chromosome is easily obtained by single crossing over between the two scutes. Since these inversions are sensibly identical except for the fact that the left break of $scute^{SI}$ is just to the right of the scute locus and that of $scute^{8}$ just to the left of it, this chromosome has two scute genes, $scute^{SI}$ near the left end and $scute^{8}$ near the right end, but is otherwise sensibly identical with either a $scute^{SI}$ or a $scute^{8}$ chromosome. In-S designates "Inversion-S" (S, Sinitskaya), an inversion slightly smaller than and in very nearly the same position as "delta 49," which arose in the $scute^{SI}$ chromosome, by irradiation, at the same time that the $scute^{SI}$ itself arose. I made up his particular chromosome for the purpose of studying mutations in the female because there is needed for this object a chromosome having (1) very ittle crossing over throughout its length, (2) suitable markers, both dominant and recessive, and (3) comparatively high viability and fertility. Condition 1 s here fulfilled by the two inversions, one included within the other, although rarely (less often, I believe, than in the case of C1B) double crossing over does occur; this usually separates the Bar from the rest, so if both Bar and apricot loci are followed, one can be virtually certain of what one has. Condition 2 has been provided by Bar and apricot. Condition 3 holds because the duplication of the scute locus largely prevents the lowering of fertility and viability that $scute^{8}$ or $scute^{SI}$ alone would give.

For the purpose of the initial detection of mutations in the X chromosome and for their subsequent testing and retention in stock, the writers have found this chromosome to be distinctly superior, for the ollowing reasons, to the C1B chromosome used widely in such experiments: Crossing over is practically eliminated throughout the ength of the X chromosome. In a test experiment involving 30 cultures in which female parents were Muller-5/scute-crossveinless-vermilion-forked-carnation, no crossovers were observed among 622 male offspring. In other cultures the rare double crossovers nvolving Bar have been seen on an average of only one in several housand flies examined, but the presence of two markers readily identified these. The stock is viable in homozygous form, and this gives it a distinct advantage over any C1B balanced stock in procuring arge numbers of virgin females for initial crosses. Homozygous Muller-5 females have a lower viability and fecundity than females eterozygous for Muller 5 and any sex-linked mutant either lethal or visible, except a few weak dominants such as "Minutes." This makes possible the retention of any new lethal or weak recessive visible indefinitely in stock in a pseudo-balanced condition with Muller 5, provided that the stocks are cultured in ½-pint bottles and that enough parents are used in each generation to eliminate the chance selection of only Muller-5 females when flies are shaken into a fresh stock bottle. Such stocks could, of course, be carried in smaller culture bottles if heterozygous females were selected as parents each generation.

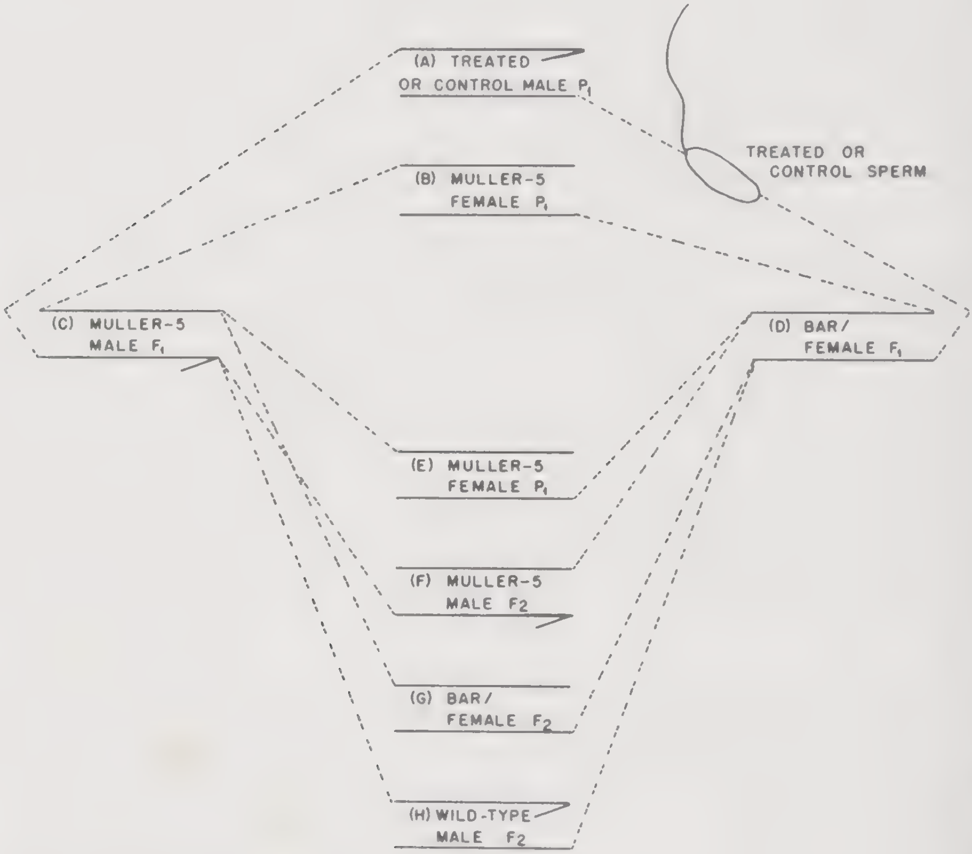

Fig. 7.1—Muller-5 technique for the detection of lethals and visibles in the X chromosome of Drosophila melanogaster (see Sec. 2.1 for full explanation).

The use of this stock in testing for either lethals or visibles that have arisen in the X chromosome of control or treated males is illustrated in Fig. 7.1. Treated or control males (A) are mated to Muller-5 females (B). The F_1 males are Muller 5 (C), and the F_1 females are heterozygous Bar (D). After the F_1 flies have been aged together for several days, each female is placed in a separate test-culture bottle with one or more Muller-5 males. Four classes of F_2 flies generally appear in such a test culture: Muller-5 females (E), apricot Bar males (F), heterozygous apricot Bar females (G) carrying one X chromosome from the control or treated grandfather, and wild-type males

H) carrying the X chromosome from the control or treated grandather. If a new lethal has arisen in the control or treated sperm cell see Fig. 7.1), all of class H will fail to appear. If a new visible has risen, all of class H will show it. A further test of the lethal or visble is made by mating females (G), which carry the new mutation, to heir Muller-5 brothers (F). The new mutation can be carried indefiitely in this manner since the Muller-5 chromosome eliminates rossing over. The occurrence of nondisjunction males among the F_2 flies will never obscure the presence of a new lethal since such nales will be Muller 5 and not wild type. The genetic setup is particlarly convenient because only at one stage in the entire procedure s it necessary to use virgin females, the F_1 Muller-5 females (B). Even at this point the inclusion of an occasional nonvirgin Muller-5 emale has no serious consequences since her homozygous apricot Bar daughters are easily separated from the red heterozygous Bar F_1 females used for the test matings. This is an important item in a arge-scale experiment.

About one-fourth the lethals and visibles found were tested for inkage relations. F_2 females (G) or F_3 heterozygous Bar females vere mated to the multiple recessive males, scute(0.0)-crossveiness(13.7)-vermilion(33.0)-forked(56.7)-carnation(62.5). Their non-Bar daughters, carrying the new mutant in one X chromosome and he recessive tester genes covering the genetic map in the other X hromosome, were mated to their Muller-5 sons. From five to seven uch pair matings were made for each gene tested, and a lower limit f 200 males to be classified was set. The use of Muller-5 fathers in hese tests again eliminated errors that might have arisen from nonisjunction males.

2.2 Choice of Stock for Testing Mutation Rate. For irradiation vork at low dosages, it was desirable to use a stock of flies with a ow control mutation rate and one that would not prove highly varible. Canton Special was tentatively chosen as the stock to be used, rovided it showed on test a low control mutation rate suspected from revious experience. The following description is taken from Bridges nd Brehme:[9]

> A standard wild-type phenotype, obtained from a stock collected in Canton, hio (selected by Bridges). The salivary chromosomes were found by Bridges be normal. The stock contains a recessive for multiple thoracic and scullar bristles, which overlaps wild type in most of the flies but appears spodically in strains in which Canton Special has been used in crosses.

The stock had originally been made isogenic by Bridges, and, about years before these experiments were started, the process had been epeated by Curt Stern.

During the autumn and winter of 1943, preliminary tests on control mutation rates in Canton Special and seven other stocks were run. As data accumulated, it became evident that Canton Special had a low natural mutation rate, considerably lower than the average of the other seven stocks and possibly as low or lower than any of the others.* The results of these tests are given in Table 7.1. The values for the seven stocks include about 3000 tests run during the spring and summer of 1944. Since Canton Special is a wild-type stock, it has the advantage over any stock homozygous for visible mutations which might mask new visibles; e.g., a stock containing white eye color would eliminate work on all other eye colors at the white and other loci. During the course of these tests a routine was gradually evolved whereby large numbers of test cultures could be run through on a uniform schedule.

Table 7.1 — Preliminary Tests on Natural Mutation Rate in Stocks of Drosophila melanogaster

Stock	Chromosomes tested	Lethals and visibles arising in male germ cells		
		Lethals	Visibles	Lethals, %
Pittsford, N. Y.	2,218	5	1	
Webster, N. Y.	1,913	3	1	
Oregon R	1,923	2	1	
Muller 5	1,366	1	0	
White	1,080	3	0	
Yellow-white-forked	764	2	0	
New Wilmington, Pa.	727	0	1	
Total	9,991	16	4	0.16
Canton Special	20,324	17	2	0.08

2.3 Culture of Muller-5 and Canton-Special Stocks. Each week on Friday, 10 or 11 ½-pint cultures of Muller 5 were started. The usual molasses-cornmeal medium, enriched with dry brewer's yeast, was used for these and all other cultures. Moldex was added to keep down mold growth. Carragar, a specially prepared extraction of the marine seaweed, Chondrus crispus, was substituted for agar because of the difficulty involved in obtaining adequate supplies of the latter. About 50 pairs of parent flies were used in each ½-pint culture. On the following Tuesday, two double sheets of Kleenex soaked in a suspension of about 100 g of baker's yeast in 600 cc of water were added to each ½-pint culture. The stocks were kept in an incubator at 24 to 25°C, and flies were emerging at this temperature 11 to 12 days after the cultures were started. Each Wednesday, virgin females to be used as parents for 1 week's supply of flies for test matings were isolated. All flies were shaken out of the stock bottles Tuesday night and collected Wednesday morning, or flies were shaken out Wednesday morning, and virgins were collected Wednesday afternoon. Each week 400 or 440 virgin females were isolated. With the culture methods outlined above, this required about ½ hr. The following record was kept to illustrate this point. From seven ½-pint Muller-5 cultures, the flies were shaken out at 10:00 a.m. Newly emerged flies were shaken out at 3:30 p.m. and etherized, and 625 virgin females, maximum age 5½ hr, were isolated by 4:00 p.m. The Muller-5 females were placed in 10 or 11 ½-pint cultures bottles, 40 flies per bottle, and aged until Friday (2 days), at which time the Canton-Special control or irradiated males were added to these bottles. On Friday, flies which had emerged the 2 previous days in the Muller-5 stock bottles were used to start fresh stocks, and the old ones were discarded. Generally these flies were simply shaken into the new stock bottles, but about once a month the parents were selected under the binocular microscope to eliminate any round-eyed males or heterozygous Bar females due to Bar reversion in the Muller-5 stock.

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*For a discussion of the variability of spontaneous mutation rates, see Muller.[10]

Stock of Canton Special was started each week on Wednesday by shaking newly emerged Canton-Special flies into six ½-pint culture bottles. On Friday these flies were all shaken into one ½-pint etherizer and etherized together. The bottles from which they had been shaken were set back as fresh stock and yeasted on Monday. The flies that had been shaken out and etherized were 2 to 4 days old, and from them 400 or 440 males were selected under the binocular microscope and placed in gelatin capsules, 20 flies per capsule, for irradiation or as controls. These males were examined to make certain that they showed no new visible mutations at the time they were isolated. The capsules were then placed in vials; each vial was marked with the radiation dosage to which the males were exposed. After irradiation, the flies were immediately placed with the virgin Muller-5 females, 40 males per ½-pint bottle; each bottle was marked with the dosage received. The Canton-Special stock was thus carried in six lines, and the flies from the six lines were thoroughly mixed and sampled for each week's lot of parent males to be irradiated or to serve as controls. The Canton-Special stocks were purposely raised under somewhat crowded conditions in order to eliminate semilethals that might arise in the stocks. Such semilethal flies are generally weaker than the wild type, and by some larval and adult crowding it was hoped that they would largely be killed off. It may be argued that crowding the Canton-Special stock might have affected in some way the natural mutation rate. This might be true, but this possible objection seemed to be outweighed by the necessity of eliminating as far as possible any semilethal males that might otherwise be used as parents for the experimental cultures.

On Tuesday the parent flies from the 10 or 11 crosses of Muller-5 females to Canton-Special males started the previous Friday were shaken into a fresh set of marked ½-pint culture bottles, and the first set was yeasted with Kleenex-yeast suspension. On Thursday the parent flies from the second set of cultures made up on Tuesday were discarded, and these cultures were yeasted.

As the F_1 flies (C and D of Fig. 7.1) began emerging, they were shaken into marked ½-pint aging bottles. These F_1 flies generally started emerging 12 days after the cultures were started, i.e., on Wednesday. Emergence from the two sets of cultures spread over a period of about 1 week. The first flies that emerged after having been aged for several days were used on the following Monday for the pair-mating test cultures of which the flies E, F, G, and H of Fig. 7.1 were the offspring. All test matings for a given week were made from the offspring of one lot of irradiated and control males. From 40 to 60 ½-pint aging bottles were used each week. It was found that the use of aging bottles cut the number of failures in the pair-mating test cultures from around 10 to less than 5 per cent. Females aged with Muller-5 males were generally already impregnated and ready to lay fertile eggs when placed in the test-culture containers. The small amount of work involved in aging the flies was well repaid in results attained.

2.4 Test Cultures. During the period from Dec. 1, 1944, to June 1945, approximately 127,000 test cultures were made up; of these, 121,353 (an average of 17,336 per month) developed and were examined for lethals without etherization (see Sec. 2.5). In the earlier part of the work, fewer test cultures were reared since they were all etherized and examined for both lethals and visibles. The following routine was followed for the period mentioned above. Test cultures were reared in 1-oz culture bottles ("creamers"). The medium was the same as that used for ½-pint cultures. Creamers were arranged in rows on tin trays; three-fourths of these trays held 95 creamers each, and one-fourth held 120 creamers. Hot medium was poured into four trays of creamers each morning and afternoon, a total of 810 creamers per day. After cooling, the culture bottles were inoculated with a drop of heavy yeast suspension, and the four trays were placed in the refrigerator without capping the bottles. The lot made up in the morning was used that afternoon, and the lot made in the afternoon was used the following morning. On Saturday, only one lot of culture bottles was prepared, and these bottles were used the following Monday morning.

When a tray of creamers was removed from the refrigerator, it was covered with cheesecloth. Flies from the aging bottles were etherized, and males and females were separated and placed in dry vials where they could easily be reetherized. One female and one or more males were placed in each creamer, which was immediately covered with a pasteboard creamer cap in which three short cuts were made with a dental scalpel for ventilation. As soon as creamers were removed from the refrigerator, the air within them began to warm up with a consequent drop in relative humidity and evaporation of moisture from the food surface. Few flies became stuck in the medium, and there was less contamination with mold than there would have been if paper or other materials had been added. Failure of a culture to develop was, however, generally due to the parent female becoming mired in the food medium, often in the spot of yeast. In order to avoid this as much as possible, the yeast was placed at one side of the creamer. Actually, many trays came through with no failures, and the percentage of failures was definitely correlated with atmospheric humidity. A library card was placed on each tray with the date and the X-ray dosage marked in two ways to avoid errors in recording. The trays were then placed in order on shelves in open cabinets in the laboratory and were not disturbed until the cultures were examined for lethals exactly 2 weeks after they were made up. Parent flies were not removed since their phenotypes were such that they did not interfere with the identification of the lethals. Thus at all times there were 88 trays and 8910 test cultures arranged in sequence on the cabinet shelves.

2.5 Examination of Test Cultures for Lethals. On Monday through Friday eight trays of test cultures per day were examined for lethals, and on Saturday four trays were examined. Since the cultures were arranged in rows on the trays, they could always be examined in the same order. On the average, more than 100 flies were present per culture. These were examined by placing the creamer on its side under the low power of the binocular microscope, magnification 7×, and checking for wild-type males, which could readily be identified at this magnification. The 810 cultures could be examined accurately in this manner in less than 2 hr. Each culture without offspring was set out of the tray and also each culture with no wild-type males, a potential lethal-bearing culture. The total number of fertile cultures and of failures was recorded on the card, and this card was set in a small tray with any potential lethal cultures or filed if there were none of these present. Potential lethal cultures were set back for 2 more days, and then the flies were etherized. Each class of flies was recorded on a lethal card together with the date on which the lethal was found. At this time two new cultures of three heterozygous apricot Bar females and five Muller-5 males were made up to test the lethal through another generation. A later count of the original lethal culture and counts of the two F_3-generation tests were made and recorded on the lethal card. In case of linkage tests, heterozygous apricot Bar females from the F_3 cultures were mated to the multiple recessive males mentioned above. The few cultures coming through with less than 12 flies were scored as failures and were not included in the total. With the food medium enriched with brewer's yeast, the number of such cultures was extremely low and could not have introduced any large error. However, to be sure that such low-yielding cultures were not due to some dominant effect of a lethal carried, many cultures of a few flies containing no wild-type males were tested through another generation using the heterozygous Bar females present. The results of such tests indicated that there was no selection against lethal-bearing chromosomes by the discard of the small group of cultures with only a few flies per culture. In such cultures, generally one or both parents were found dead, thus explaining the low yield. The procedure outlined above was specifically designed for the discovery of pure lethals, which experience had shown was by far the largest class of mutants that could be recovered rapidly and with little subjective error. No attempt was made to save semilethals, and a culture was discarded as soon as one wild-type male was seen. Since such a male could not be due to nondisjunction and since crossovers were practically eliminated, no pure lethals would be discarded on this criterion. All the checking for lethals without etherization of flies was done by Warren P. Spencer.

Until mid-July 1944, all control and radiation test cultures were etherized and examined under the binocular microscope for both lethals and visibles. Since this method required much more time, all three workers helped. During this period another step was included in the routine. About 5 days after the test cultures were made up, parent flies were removed, and a small piece of Kleenex paper soaked in yeast suspension was put in each creamer. This increased the yield somewhat, and the presence of the paper made it easy to shake out adult flies at the time of etherization since, especially in warm weather, carragar medium becomes soft and it is difficult to shake flies from the culture. Lethals and visibles found were tested in the same way as described above for lethals.

During the period December 1944 to July 1945, M. Harland also examined most of the cultures from flies receiving radiation of 150 r or more for visibles after they had first been checked for lethals. These cultures were etherized in checking for visibles. Problems involved in scoring both lethals and visibles are discussed below.

2.6 *X-ray Treatments.* In an ideal radiation experiment all lots of flies receiving different dosages would be irradiated on the same apparatus on the same day, and the output of the apparatus would be checked during the radiation with a dosimeter that had been standardized immediately before the radiation. Obviously these conditions could not be adhered to in a large-scale experiment involving eight different dosages and hundreds of thousands of test cultures. It was impossible to predict at the beginning of the experiment the number of points on the dosage curve that could be profitably investigated. It has been the aim in these experiments to spread the low dosages over as many lots of flies and as much time as practicable and to spread the control lots over the entire period of the experiment.

Table 7.2 — Dosages and Other Conditions of Radiation of Lots of Males Irradiated at Different Dosages

Dosage, r	Number of weekly lots	Voltage, kv	Current, ma	TSD, in.	Dosage rate, r/min
Control	70				
25	43	90	3.0	18.5	15 – 22
50	11	90	3.2		10
50	45	90	3.0	18.5	15 – 22
150	12	90	3.2		10
150	31	90	2.0	18.5	15 – 22
500	14	90	3.0	18.5	15 – 22
1000	5	90	3.2		10
1000	20	90	3.0	7.25	91 – 100
2000	2	90	3.0	7.25	91 – 100
3000	2	90	3.0	7.25	96
4000	1	90	3.0	7.25	96

A lot refers to control or irradiated flies used for 1 week's test cultures. These may therefore be referred to as "weekly lots." Table 7.2 gives the number of weekly lots for each dosage. The weekly lots at 50, 150, and 1000 r given at 10 r/min were irradiated on a machine whose output was checked with a dosimeter at 6-week intervals. In all, these lots account for 7535 test cultures at 50 r, 13,595 test cultures at 150 r, and 3938 test cultures at 1000 r. For all the other lots, which include the bulk of the work, dosimeter readings were taken each week at the time the flies were irradiated. Since this work was a small part of a much larger radiation program, the dosimeters were being checked from time to time for accuracy. In spite of this fact, small errors in dosimeter readings occurred. It is felt that these errors would tend to be equalized by the large numbers of treatments given at dosages from 1000 r down. Data obtained at dosages above 1000 r had relatively little bearing on the main problem being investigated. At the beginning of the experiment and before any flies were irradiated, about 20,000 Canton-Special control cultures were reared but not recorded in weekly lots.

2.7 Mutational Category Chosen for Quantitative Study. It seems pertinent at this point to consider briefly the kinds of mutations occurring in Drosophila and their adaptability to a quantitative study of the type undertaken and to define carefully the category of mutation chosen.

Mutations occur in both autosomes and sex chromosomes, and there is abundant evidence that more mutations occur in the autosome complex than in the X chromosome. However, there is no breeding procedure by which newly arisen autosomal mutants can be found as easily and effectively as the sex-linked mutants by the method outlined in Fig. 7.1. Although the data are as yet fragmentary, it would seem that comparative studies on sex-linked and autosomal mutation should make possible a transfer of findings on X-chromosome mutation rate to the mutation picture in the autosomes.

Mutations are known to occur at different stages in the development of the organism. The study of mutation rate in mature sperm has certain technical advantages in obtaining large quantities of valid data. In extensive experiments on natural mutation rate, Muller[11,12] has shown that over-all mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's preimaginal life, than in those of a week later derived from a nonvirgin male. It should be noted that all the Canton-Special spermatozoa used in the control and radiation experiments reported in this chapter were from males 1 week or less old.

Mutations may be somewhat arbitrarily grouped into the following overlapping categories: dominant lethals, recessive lethals, recessive lethals with visible dominant defects, sterility mutants, recessive semilethals without visible effects, recessive semilethals with visible effects, deleterious or lowered viability mutants without visible effects, deleterious or lowered viability mutants with visible effects, recessive visibles, dominant visibles, and isoalleles (mutants whose presence can be detected only by special genetic or environmental tools).

Catcheside and Lea[13] review the data on dominant lethals in Drosophila melanogaster and present new data. Although these data can be shown by statistical means, using relatively high X-ray dosages, to have a higher incidence than any of the other categories quantitatively studied, their use in experiments at low dosages is out of the question because of relatively high control variables. Furthermore, although they are well adapted to the study of certain theoretical problems, their significance can hardly be compared with that of certain of the other categories.

Isoalleles of the normal alleles present in the original stock may, and probably do, constitute the largest group of genetic changes, but the obvious difficulties in demonstrating their presence eliminate them from consideration. Another large group of mutations is represented by genes with lowered viability. Their occurrence was first noted by Muller,[14] and their abundance was proved by Muller,[15] Kerkis,[16,17] and Timoféeff-Ressovsky.[18] These men came to the conclusion that the class of viability mutants is from two to three times as numerous as the lethals. More recently, Ives[19] and Dobzhansky and Spassky[20] have reported similar results. This heterogeneous group of genetic changes, readily influenced in expression by genetic and environmental modifiers, is not amenable to quick detection and objective classification and is therefore not favorable for the type of quantitative study reported here.

Visibles, both dominant and recessive, occur in much smaller numbers than any of the previously discussed categories or than pure lethals. Furthermore, they are subject to the traditional objection that their discovery depends on the training of the investigator and is less objective than the discovery of pure lethals, in which the proper genetic tools are used. In any case the time involved in finding them is very much greater than that in finding pure lethals.

This leaves lethals and semilethals with and without visible effects. Unfortunately for a purely objective study, there is a sizable borderline group between the pure lethals and semilethals. In this group are found mutants that may in one generation or culture appear to be pure lethals but that, in another generation or culture either by the vagaries of statistical sampling or through changed culture conditions, may appear to be semilethals. About half or a little over half of the extreme semilethals found show visible effects. This visible effect forms an objective criterion for classification. The other semilethal group grades into the lowered viability class, and an arbitrary criterion of classification needs to be set up to separate them. Such criteria are either not accurate because of varying numbers of flies per culture, or, if accurate, they are laborious and time-consuming, an important factor to be considered.

Second-generation tests of all lethals found were also made. In spite of the fact that 65 of them appeared as pure lethals in the first-generation count but as semilethals in the second-generation count, they have been included with the 914 lethals which behaved as pure lethals in both generations, and the calculations were based on the total number of 989 lethals of which a small number behaved as semilethals in the second generation. This has been done because the criterion of selection in the first generation has been a purely objective one, i.e., the total absence of all wild-type males. For reasons stated in this section, those semilethals with visible effects, the condition of a few flies appearing in the first generation, have not been included.

3. LETHAL MUTATION RATE IN CONTROL AND IRRADIATED MALES

Table 7.3 presents the data obtained during the course of the experiment on lethal mutation rate in controls and males irradiated at the several dosages mentioned in Sec. 1. In the first column the dosages are given, in the second column the number of test cultures reared following each dosage, and in the third column the number of tested chromosomes carrying at least one lethal. If lethals are produced at random throughout the test material, it is clear that, at high dosages and high mutation rates, some chromosomes will have two lethals induced simultaneously. It has been pointed out by Gowen and Gay[21] that the apparent mutation rate at high dosages will therefore fall below the value expected on the linearity rule unless corrections for test chromosomes carrying two or more lethals are made. Obviously the chief interest here is in mutation rate at dosages where this correction factor will be negligible. However, at the high values, 1000 r and up, no attempt has been made to introduce this correction factor for the following reason: It must be remembered that, except where linkage tests have been made, the breeding procedure described above has been such that the mutational complex of a chromosome is not separated into its constituent components. This is due to the fact that all crossing over is eliminated. Therefore at the higher dosages the observed number of apparently pure lethals will be somewhat increased by cases where two semilethals or lowered viability mutants present in the same chromosome cause no males of the class carrying the chromosome in question to appear even though some would have come through if either one of the semilethals had been present alone. This might be due to an interaction and lowered viability effect of the two semilethals in the same organism. It might also result from the lowered chance of observing one individual in the sample represented by the flies in one test culture. For example, if 50 wild-type flies were expected in a culture, then with two semilethals, each with a viability of 10 per cent of normal, the number of flies of this class expected would be 0.5; and with two lowered viability mutants, one with 30 per cent and the other with 20 per cent of normal viability, the expected number of flies coming through would be 3. Obviously, in some individual cases with this expectation, none would actually emerge. Since semilethal and lowered viability mutants together make up a group of considerable size, the observed mutation rate at high dosages will thus tend to be increased. Since the above factors act in opposite directions, although not necessarily canceling one another, it is considered unprofitable to attempt to introduce a correction factor where the highest dosage used is 4000 r. It is of interest to note in the paper of Gowen and Gay[21] that, of the six points on each of two curves for the percentage of chromosomes without recessive lethals, the last two points (highest radiation) fall below the fitted curve. Such a result is not surprising when a correction was made for chromosomes bearing two lethals and no counter correction was made for double semilethals and double lowered viability mutants.

Table 7.3 — Summary of Data on Lethal Mutation Rates for Controls and for Different Dosages of X Rays

Dosage, r	N*	n*	Mutation rate,† %		
Control	73,901	72	0.0788	0.0974	0.1220
25	51,907	88	0.1396	0.1695	0.2080
50	31,560	77	0.1985	0.2439	0.3034
150	23,195	74	0.2586	0.3190	0.3983
500	6,634	87	1.0802	1.3114	1.6087
1000	6,977	147	1.8102	2.1069	2.4667
2000	2,755	130	4.0276	4.7187	5.5691
3000	2,029	132	5.5535	6.5057	7.6737
4000	1,843	182	8.6462	9.8752	11.3391

*N, total number of chromosomes tested; n, total number of chromosomes in which lethals were found.

†Column 5 gives the lethal mutation rate observed in percentage, and columns 4 and 6 give the percentage values of which the observed mutation rates would be the upper and lower 5 per cent fiducial limits (5 per cent in both tails jointly).

Column 5 of Table 7.3 gives the lethal mutation rates expressed in percentage of lethal-bearing chromosomes found in the tests for controls and at different dosages. It was intended to proportion the test cultures at different dosages in such a way that the standard error would be about the same percentage of the mutation rate for each dosage and for the controls. Actually on this criterion about twice as many data have been obtained at dosages from 1000 r up as at the lower dosages and controls. No attempt was made to obtain exactly the same number of mutants or to keep the ratio of standard error to mutation rate constant for all dosages studied because this would inevitably have resulted in selective error toward the end of the experiments.

Columns 4 and 6 give percentage values of which the observed lethal mutation rates would be the upper and lower 5 per cent fiducial limits (5 per cent in both tails jointly). Thus, if 0.0974 per cent represents the observed control mutation rate, then a true rate of 0.0788 would be expected to result once in 40 times in an observed value as large as or larger than 0.0974. Similarly, a true rate of 0.1220 would be expected to result once in 40 times in an observed value as low as or lower than 0.0974.

The calculated values were obtained by the following method suggested by Donald R. Charles, in which n'/N represents a "universe" mutation rate and n/N represents the mutation rate in a sample of N. The standard error of n' is

$$\sqrt{n' \left(1 - \frac{n'}{N}\right)}$$

By ordinary theory the upper 5 per cent fiducial limit of n' is $n' + 1.96\epsilon$, but from Stevens[22] this limit is actually $n' + 1.96\epsilon + C$, where C is a correction. Here the problem is to find an n' such that

$$n' + 1.96\epsilon + C = n$$

For any particular sample, n and C are known; the equation can thus be solved for n.

On the basis of the experimental evidence it would seem that an X-ray dosage even as low as 25 r produces a significant rise in mutation rate over that found in the controls.

Figure 7.2 shows the fit of a straight-line curve to the data obtained. The vertical lines represent the statistical limits at the 5 per cent level of significance. The curve is based on all the data reported in Table 7.3, which give a control mutation rate of 0.1004 per cent per chromosome tested and an induced mutation rate of 0.00215 per cent per roentgen of radiation per chromosome tested. Only the control and 25-, 50-, and 150-r points are plotted on this curve. On the

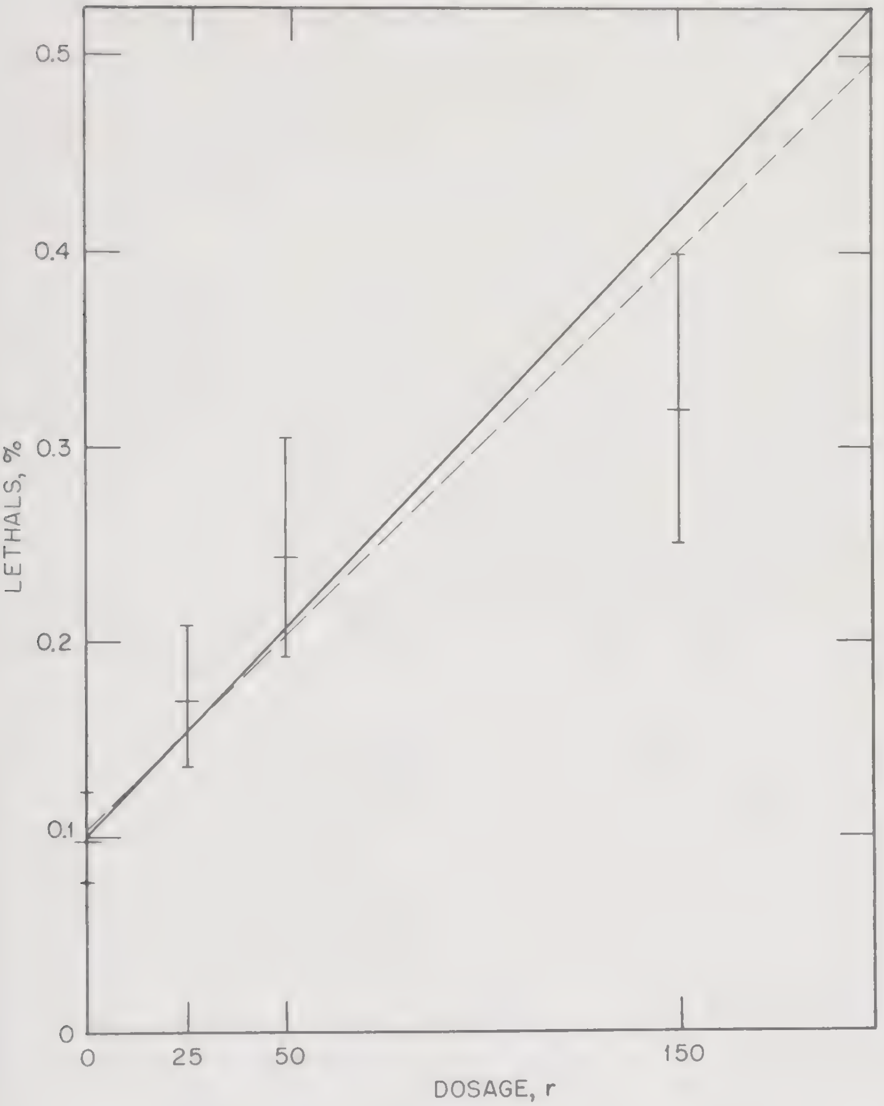


Fig. 7.2—Linear relation between dose and lethal mutation rate. ——, based on all data in Table 7.3. - - -, based on the control to 1000-r data, omitting the 2000-, 3000-, and 4000-r data. The data points for the control and the 25-, 50-, and 150-r doses are shown as small central horizontal marks. The ends of the vertical lines represent the upper and lower 5 per cent fiducial limits of the values observed. Calculated mutation rates (maximum likelihood) from all data: control, 0.1004 per cent; induced, 0.00215 per cent per roentgen. Calculated mutation rates (maximum likelihood) from 0 to 1000 r: control, 0.1039 per cent; induced, 0.00197 per cent per roentgen.

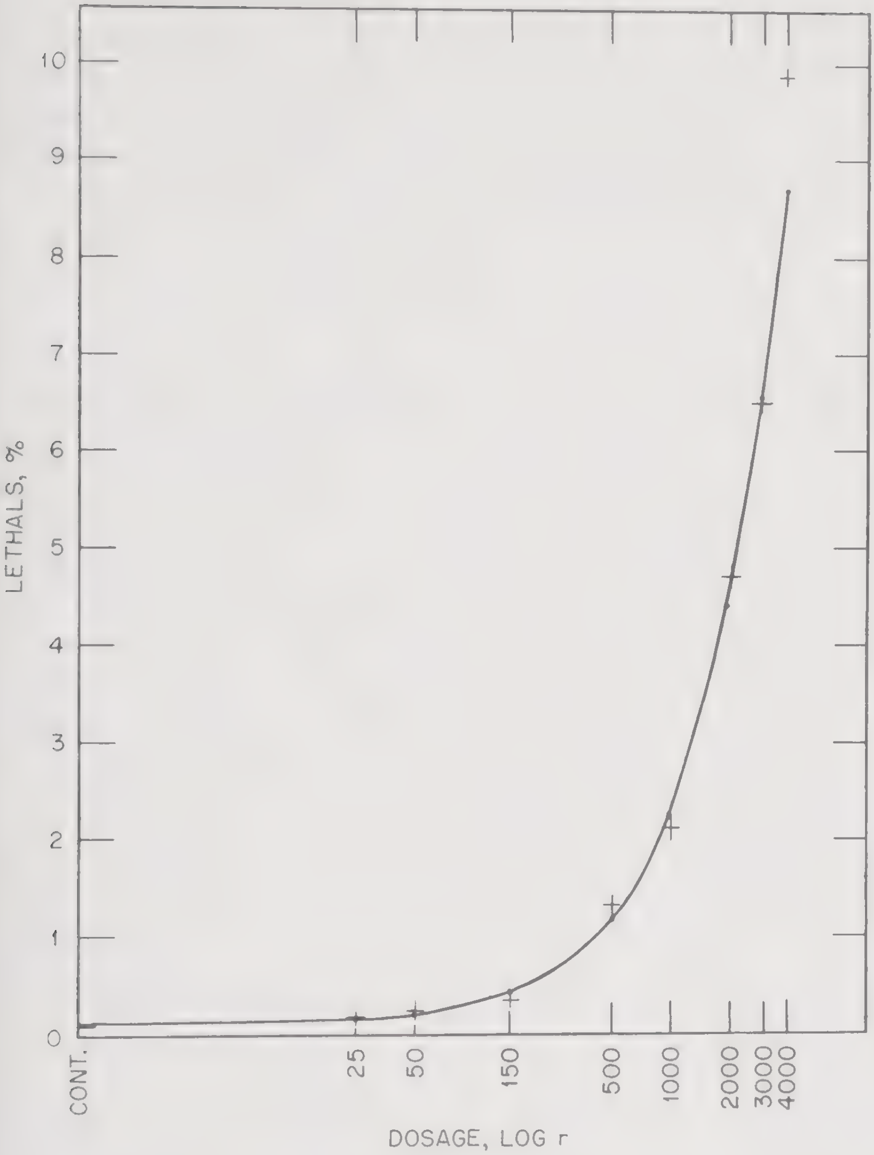
basis of this graph it is not unreasonable to conclude that the lethal mutation rate in mature sperm of the Canton-Special stock is directly proportional to the X-ray dosage for values as low as 25 r. The low value at 150 r must be considered in connection with the entire experiment. Since eight dosages and control values have been studied, it is not surprising to find one point on the curve considerably out of line. Figure 7.3 is a graph of log dose vs. mutation rate made up from the data in Table 7.3.

4. CONTROL VALUES AND LETHAL CLUSTERS

At X-ray dosages of 1000 r and up, the induced mutation rate is relatively so much higher than the control rate that the latter plays a small part in the experimental procedure and the interpretation of data. This is by no means the case at low dosages in the range of 25 to 50 r where the control mutations may equal or exceed in number those produced by the radiation. It is therefore important to collect a large body of control data, in so far as possible, at the same time and under the same environmental and genetic conditions as the radiation data in order to reduce errors from control fluctuations to a minimum.

The somewhat lower than average mutation rates found in both control and irradiated flies need some explanation. Possibly the genetic background in Canton Special is such that certain mutations which would act as lethals in some stocks behave as semilethals in these experiments because of the general vigor of the stock. Many of the experiments on the lethal mutation rate have been performed on multiple mutant stocks less vigorous than Canton Special. Furthermore, in these experiments the use of yeast-enriched media tended to permit the survival of some flies that on a poorer medium would all have died before the adult stage. These factors would contribute in the same degree to the lower than average mutation rate in both control and irradiated animals. They do not account, however, for the fact that, although the natural mutation rate is low, the radiation data indicate that it would be doubled at about 50 r rather than at the somewhat lower dosage previously calculated by Timoféeff-Ressovsky.[5] Whether this apparent deviation from the results of other workers, if significant, is due to intrinsic causes or to differences in dosimetric measurements cannot be decided at this time.

The data on both controls and each dosage group were collected and filed in weekly lots as explained above. These lots were in turn made up of sublots consisting of the test cultures on each tray, an average of a little less than 100 cultures per tray. The filing of the data in this way made it possible to observe and to test cases in which



ig. 7.3—Graph of log dose vs. mutation rate, constructed from all the data Table 7.3. The crosses above or below the theoretical points on the curve ark the actual mutation rates observed at the different dosages.

e lethal distribution among control and low-dosage cultures ap-eared to be nonrandom. This procedure led to the discovery of sev-ral cases designated as "lethal clusters" among the controls and the w-dosage tests. Since this matter has an important bearing on the llection of data in low-dosage experiments, some specific cases ill be presented in detail.

Among 1726 test cultures examined in the forty-second lot of controls, seven lethals were found where, on the basis of the control rate established, about 1.7 lethals were expected. Four of the lethals were found in two trays, two per tray, and all were late pupal lethals. Since not more than 20 per cent of all lethals are pupal, this made their chance association even less likely. It was thought that these might have come from a Canton-Special male carrying a semilethal X chromosome, but subsequent tests showed that this was not the case. In Table 7.4 the data are presented. It will be noted that the linkage tests placed the loci of these four lethals at 22.7, 25.3, 23.2, and 23.4 on the genetic map of the X chromosome. This fact, in conjunction with their phenotypic character (late pupal), clearly indicates that they are one and the same lethal. Since there were three creamer cultures of each lethal reared, this really amounted to 12 test cultures and 1333 flies reared with not a single wild-type male appearing. In addition, a ½-pint mass culture of each of the four lethals was reared, and several hundred flies from each culture were examined with no wild-type males appearing. It seems well established that this was a pure lethal and not a semilethal and that the four lethals

Table 7.4 — Lethal Cluster in the Forty-second Lot of Controls*

Cultures made up	Tray No.	Number of cultures	Number of lethals	Number of flies (linkage test)	Locus
12/14/44	8	91	2	(a) 340	22.7
				(b) 335	25.3
12/14/44	9	90	2	(a) 332	23.2
				(b) 248	23.4

Counts Demonstrating That These Are Pure Lethals
(One F_1 and Two F_2 Cultures)

Lethal	Date	Apricot Bar ♂♂	+♂♂	Apricot Bar ♀♀	+/Apricot Bar ♀♀	Total
8a	12/30	79	0	78	162	319
8b	12/30	76	0	77	191	344
9a	12/30	68	0	68	185	321
9b	12/30	94	0	105	150	349
Total		317	0	328	688	1333

*For full explanation, see Sec. 4; for locus determinations, see Secs. 2.1 and 7.

ound actually came from four sperm cells derived from the same Canton-Special male and all carrying the same lethal which must ave arisen in the germ tract of this male. The male might possibly ave been a gonosomic mutant (Sidky[23]), with the germ cells all lethal earing and the body cells nonlethal bearing. Such an explanation is, n principle, the same as that suggested, the lethal mutation having risen at an earlier stage in the ontogeny of the male.

Table 7.5 — Lethal Cluster in the Forty-second Lot of 50 r

Cultures made up	Tray No.	Number of cultures	Number of lethals	Number of flies (linkage test)	Locus
3/6/45	3	119	3	(a) 119	38.4
				(b) 143	38.9
				(c) 267	37.9
3/6/45	4	89	4	(a) 258	36.6
				(b) 205	0.0
				(c) 234	39.5
				(d) 207	38.4

Counts Demonstrating That These Are Pure Lethals
(One F_1 and Two F_2 Cultures)

Lethal	Date	Apricot Bar ♂♂	+♂♂	Apricot Bar ♀♀	+/Apricot Bar ♀♀	Total
3a	3/20	89	0	76	90	255
3b	3/20	103	0	79	128	310
3c	3/20	92	0	80	144	316
4a	3/20	142	0	108	183	433
4b	3/20	122	0	91	154	367
4c	3/20	125	0	111	184	420
4d	3/20	135	0	131	164	430
Total*		686	0	585	893	2164

*4b omitted.

Among 804 test cultures examined in the forty-second lot at 50 r, 2 lethals were found. At the mutation rate established for this dosge, about two lethals were expected in the 804 cultures. Three of ese lethals were found in one tray of 119 cultures and four in a tray 89 cultures. These seven lethals were run through linkage tests, d three cultures of each were raised. All proved to be pure lethals, d the linkage tests showed that six of the seven were probably idencal. The data on this case are given in Table 7.5. The other five lethals in this lot were not tested for linkage relations. Some or all of them may have been identical with the six shown to be the same. Such a cluster of pure lethals can best be accounted for by assuming that a lethal arose early in the germ tract of one of the 80 Canton-Special males used as grandparents of the flies in the 804 test cultures. These lethals would then be control lethals and not due to the radiation, although they appeared in the 50-r tests.

In the forty-sixth lot at 50 r, three lethals appeared in one tray of 89 test cultures. Linkage tests placed their loci at 0.0, 28.0, and 30.4. Apparently two were identical; the third was at a different locus. Two lethals appeared in a tray of 94 cultures in control lot 67. They proved to be identical; the linkage tests placed their loci at 29.1 and 29.2. In the third lot at 50 r, two lethals were found in a tray of 89 cultures; the loci of both by linkage tests proved to be at 52.0. In the fifth lot at 150 r in a tray of 94 cultures, two mutants were found; one gave no wild-type males in the test culture, and the other gave one. The linkage tests placed these at 23.7 and 24.4, respectively. During the linkage tests a few flies came through showing extreme rough eyes, coarse and irregular bristles, and coarse-textured wings sometimes nicked. This is apparently an extreme semilethal allele of goggle, locus 25 in the X chromosome (Bridges and Brehme[9]). It is unlikely in the latter case that one of the Canton-Special males irradiated at 150 r was goggle since these flies were examined for mutants, and such an extreme phenotype would not seem likely to have been overlooked. It seems more likely in the light of evidence from the other cases that this represented a visible cluster.

The following case, however, illustrates the fact that a visible might be overlooked in selecting Canton-Special males for irradiation. In lot 54 at 150 r, out of 830 test cultures 14 were found showing wavy wings. These cultures were found in four trays out of the nine 150-r trays for that week. There were 354 cultures in these four trays. On the average about 10 test cultures should come from one male fly. In this case it seems probable that all 14 female parents of these test cultures showing wavy wings had obtained their wavy-bearing X chromosomes from the same male. Since some wavy males approach the normal in phenotype, it could easily have been overlooked when the Canton-Special males were selected for X-raying. This case, however, may represent another visible cluster. The linkage test of the mutant placed it at locus 40.7. The mutant fits the description of wavy, locus 41.9 (Bridges and Brehme[9]).

From the evidence presented above, it seems clear that mutation clusters, i.e., groups of germ cells bearing an identical mutation that has arisen at an early stage in the germ tract, are likely to form an appreciable proportion of the control or natural mutations recovered. The time at which the mutation occurs in the germ tract need have no bearing per se on mutation rate, but it will have a definite bearing on the structure of samples taken from mature germ cells. In small Drosophila populations this phenomenon could play a role in the high incidence of certain mutant genes. In this problem it has a definite bearing on the method of sampling the germ cells from a stock of flies in which mutation rate is being studied. In this study, between 20,000 and 25,000 Canton-Special males were used to provide the sample of sperm cells for the 200,801 test cultures. If the experiment were to be repeated on the same scale, about four times this many males would probably be used. Ideally, one male should be used for each F_2 test culture. However, this would practically double the work so that half as many data could be collected in the same time. A reasonable balance should be made between the work involved in the sampling method and that applied to the collection of test data so that the sampling errors may be kept small and the data obtained may be as extensive as possible. It seems clear in this experiment that each lethal test culture found in a demonstrated lethal cluster must be scored as a separate lethal since each test culture from a nonlethal cluster of germ cells is scored as a separate nonlethal. Obviously there is no method of demonstrating the test cultures forming nonlethal clusters, but by implication they must be there.

It seems that the amount of data presented in this chapter is sufficiently large so that the sampling error from control lethal clusters does not seriously invalidate the conclusions drawn. It is not unlikely, however, that some of the variations in control mutation rate in different stocks and in the same stock at different times as reported by various workers can be accounted for on the basis of insufficient or faulty sampling. Even when linkage tests did not demonstrate any lethal cluster, the inclusion of a disproportionately high number of germ cells from nonlethal clusters in one experiment might give a mutation rate lower than normal. It seems clear that, when control data are needed, the method of sampling the germ cells must be given careful consideration. Luria and Delbruck[24] discuss the sampling problem involved in determining mutation rate in bacteria, and Auerbach and Fano[25] consider the same problem in dealing with the scoring of mutations arising from chemical treatment of Drosophila larvae.

5. MUTATION RATE AT LOW DOSAGES

As seen from Table 7.2, only two weekly lots of flies were irradiated at 2000 and 3000 r and only one at 4000 r. Since much work has already been done at these dosages, the purpose here was only to obtain some additional data at these dosages as a general check on the low-dosage experiment. From all data obtained, assuming a linear relation of dose to mutation rate, the control mutation rate has been calculated as 0.1004 per cent and the mutation rate per roentgen as 0.00215 per cent. From these data, it appears that the control or natural mutation rate is doubled by a dose of 46.7 r.

Owing to the possibility of errors at the high dosages due to the small number of irradiated lots at these dosages and consequent chance of errors in dosimeter readings and to the inaccuracies introduced by multiple lethals and semilethals in some chromosomes, a point discussed above, it was decided to calculate the control and radiation mutation rate on the basis of the data collected at 0 to 1000 r of radiation. These data were less likely to be subject to errors in dosimeter readings since all radiations at 1000 r and lower were carried out on many weekly lots of flies (see Table 7.2). They were relatively free from errors due to multiple mutations in chromosomes tested, owing to the low induced mutation rates. Calculations on the basis of these data gave a control mutation rate of 0.1039 per cent and a mutation rate per roentgen of 0.00197 per cent. These values indicate a doubling of the control mutation rate at a dosage of 52.7 r. The two sets of values check closely, the low induced mutation rate at 150 r and the high rate at 4000 r accounting largely for the minor differences in the values.

In Fig. 7.2 the solid line represents the curve based on the assumption of a linear relation of dose to mutation frequency and calculated from all the data, whereas the broken line represents the curve calculated from the data for controls through 1000 r of radiation. On the basis of this curve the 150-r mutation value shows a deviation from the expected at about the 5 per cent level of significance, and the 50-r value shows a deviation in the opposite direction and almost as great. If the control lethal clusters reported for the 50-r material had been present in the 150-r cultures, the deviation of these two points from the fitted curve would have about the same values. The deviation of the mutation rates at the different dosages from the expected values may well be explained as due to chance, particularly with the factor of control lethal clusters taken into consideration. An examination of Fig. 7.3 and of published curves on the linear dose-to-mutation relation at high dosages will show that here also chance and unknown variables cause considerable fluctuations from the theoretical values to be expected. On the basis of control through 1000-r data the facts are consistent with the interpretation that the linear relation of dose to mutation frequency holds at low dosages.

Since these data, either including or excluding the high-dosage material, give a lower control mutation rate than most of the data published and at the same time a higher dosage necessary to double the control rate, 50 r in contrast to the 35-r estimate of Timoféeff-Ressovsky,[5] it is of importance to consider possible factors that may account for the discrepancies.

Consider first the relatively low control mutation rate obtained in these experiments. A survey of the earlier literature on the induction of mutations by X rays shows that, in individual radiation experiments, almost invariably inadequate data were obtained to establish a control mutation rate within reasonably narrow limits. This is no reflection on early workers since the high dosages employed showed a tremendous increase in the induced mutation rate even if the control rate were in error by a factor of 10 or more. However, when the small lots of control data from separate experiments are combined into larger lots representing control data collected at about the same time in the radiation work, an interesting comparison may be made. The earliest control data (reported by Muller and Altenburg[26]) give a very high mutation rate. The summary of control data (Schultz[3]), excluding the early data of Muller and Altenburg and based on 15 different experiments totaling 26,145 X chromosomes tested, gives a control rate of 0.18 per cent. Dubinin[27] published a table on control mutation rates in which two sets of data are on a scale sufficiently large to be of some validity. In this table, data of Timoféeff-Ressovsky published in 1940 on 8 lines of flies and 68,221 chromosomes tested give a mutation rate of 0.14 per cent, and data of Demerec[28] on 12 lines and 13,612 chromosomes tested give a mutation rate of 0.10 per cent. It is quite possible that improvements in culture conditions, particularly the addition of brewer's yeast to the culture medium, have resulted in the apparent lowering of control mutation rates by bringing through a sizable group of semilethals that would be scored as lethals under poorer culture conditions. The criteria used in scoring lethal mutations will also have a bearing on the apparent lethal mutation rate, and these criteria should be carefully stated for comparative purposes.

Whether the present low control rate is due to improved culture conditions and rigid scoring of lethals or to a true low rate of the Canton-Special stock or to both, a doubling of the control rate is still obtained at about 50 r either including or excluding the high-radiation data. The discrepancy between this value and that of 35 r, based on previous high-radiation experiments, may be explained by a large-scale experiment recently reported by Muller.[12] He finds that "experiments involving tests of some 200,000 X chromosomes for 'spontaneous lethals' demonstrate that the time rate of mutation varies greatly among different types of normal cells, representing different stages in the germinal cycle. Over-all mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's preimaginal life, than in those of a week later, derived from a nonvirgin male."

It seems likely that much of the earlier control data were collected from flies of varying ages, whereas the data presented here come exclusively from flies of 1 week or less in age. Since the figure for the doubling of natural mutation rate at 35 r was based exclusively on relatively high-radiation data, the control mutations occurring in the irradiated material would have a negligible effect on the results. Muller's experiments, carried on at the same time and on about the same scale as those reported in this chapter, make possible an explanation of the apparent discrepancy between the findings reported in this chapter and those reported previously. It must be concluded, however, on the basis of both high- and low-dosage data, that Canton Special has a somewhat lower induced mutation rate than the average of stocks previously tested unless the discrepancy is due entirely to improved culture conditions and rigid scoring of lethals.

The data accumulated by the writers and their associates clearly indicate that, for radiation with X rays, dosages as low as 25 r produce mutations as drastic in their effects and in the same proportion to the dosage as do exposures to high dosages. Thus there appears to be no tolerance dose below which mutations are not induced. The total effect of X rays on the induction of point mutations in a fruit-fly population will therefore depend on the mean value of roentgens to which the individuals of the population are exposed. In other words the total effect on the germ plasm of a population of 100,000 individuals would be as great if each individual received 1 r at the beginning of the reproductive period as it would be if 1000 individuals each received 100 r and the others none. If the data on flies can be shown to have transfer value to man, then widespread exposure of large numbers of human beings during the reproductive period even to low dosages of X rays must be looked upon as a serious hazard. It should also be kept in mind that, for all practical considerations, mutation of the germ plasm is an irreversible process. The new mutation, once it occurs, cannot be cured or alleviated. Since most transmitted mutations, however deleterious their effects, are recessive, the importance of the induction of a mutation is not to be reckoned in terms of its effect on one individual but rather in respect to its possible effect through transmission to many individuals over a large time scale.

6. VISIBLES AND LETHAL-VISIBLE RATIOS

Table 7.6 contains the data on the occurrence of pure lethals, semilethals without visible effects, semilethals with visible effects, and visibles. No data are given for the flies subjected to the 25-r dosage since none of these cultures were examined for visibles. In the table the pure-lethal class contains those mutants tested through two generations without showing any wild-type males. In addition to those listed, there were 78 mutants of this class found in the 25-r lots. In testing these lethals, 253,773 flies were etherized and classified, an average of 277.6 flies per lethal tested. The semilethals without visible effects are, for the most part, cultures that were set back because no wild-type males had emerged when they were first examined and in which no wild-type males were present when the first counts were made on them. Many semilethal and lowered viability mutants were observed but not recorded for the reasons given above. Therefore the figures on this group should not be taken as a quantitative measure of the total numbers of this type of mutant occurring. They

Table 7.6 — Lethal-Visible Ratio at Different Dosages

Dosage, r	Lethals (pure)	Semilethal nonvisibles	Semilethal visibles	Visibles	Corrected value* for visibles and semilethal visibles	Ratio of lethals to visibles
4000	1,843†	1,843	1,843	1,627	33.3	4.9 : 1
	20‡	20	11	22		
3000	2,029†	2,029	2,029	1,871	24.1	5.2 : 1
	125‡	8	8	24		
2000	2,755†	2,755	2,755	1,472	20.9	5.7 : 1
	119‡	15	12	5		
1000	6,977†	6,977	6,977	1,206	31.0	4.4 : 1
	137‡	10	14	3		
500	6,634†	6,634	6,634	6,328	14.3	5.7 : 1
	81‡	5	5	9		
150	23,195†	23,195	23,195	17,099	14.1	4.8 : 1
	67‡	9	6	6		
50	31,560†	31,560	31,560	7,535	7.2	10.3 : 1
	74‡	4	3	1		
Control	73,901†.	73,901	73,901	36,319	16.1	4.3 : 1
	70‡	7	10	3		
Total	836	78	69	63	159.0	5.2 : 1

*The values in this column were derived from the expression $[(N - Lv)/N'] + slv$, where N = total cultures examined for lethals, N' = total cultures examined for visibles, L = total cultures in which lethals or semilethals of either kind were found, v = visibles found, and slv = semilethal visibles found.

†The entries in this row are the number of cultures examined.

‡The entries in this row are the number of mutants found.

are included in the table to complete the record. If temperature and other culture conditions were held constant and if test cultures were always examined the same length of time after they were made up, semilethals could be recorded objectively and used in the totals for determining comparative mutation rates. However, the test cultures were subject to the usual temperature fluctuations found in a laboratory that is not air-conditioned, and, for reasons explained above, this class of mutants was not dealt with quantitatively.

All semilethals showing visible effects were classed with the visibles in calculating total visible mutation rate. This would seem to be the most objective procedure because it is possible on the basis of viability to arrange a continuous series of visibles ranging from extreme semilethal types at one end to mutants with a viability equal to most wild stocks at the other. A criterion for separating lethals from semilethals was set as the inability of any flies to emerge from the pupa case. If flies can be shaken out of the culture bottles, they are considered nonlethal even though they may die soon after emergence. Several interesting types of this sort have been seen. In the fortieth lot at 25 r, two semilethal visibles of particular interest have been found. In one the tarsal joints were absent from all legs. In another the flies showed, on emergence, complete lack of muscular coordination and soon died.

Since during the second year of work most of the cultures were not etherized and examined for visibles, it has been necessary to estimate the number of visibles occurring from the sample of cultures that were examined. In all, 73,457 cultures have been etherized and examined for visibles in addition to those cultures etherized as potential lethals and found to contain semilethal visibles. As may be seen from Table 7.6, the estimates of lethal-visible ratios at the different dosages are not based on samples of equal significance. However, the total data on visibles seem sufficiently extensive to justify their inclusion. Based on a total of 836 pure lethals and 159 visibles (of the latter 132 were discovered and the others calculated from the samples of cultures actually examined), the over-all lethal-visible mutation ratio is 5.2 to 1. Furthermore the recorded ratios are quite similar for controls and the different dosages. Since this result is subject to a considerable statistical error, the ratios may be intrinsically somewhat different. Such a difference would be expected since it is known that X-ray-induced lethals consist of two classes, namely, cytologically visible small deficiencies and cytologically unrecognizable changes, whereas spontaneous lethals are composed nearly exclusively of the latter group. It will be noted that the ratio of 10.3 to 1 in the case of the 50-r dosage is based on a relatively small number of cultures examined considering the low rate of expected mutation. Schultz[3] tabulates the lethals and visibles found in radiation experiments by five different workers. They found 1189 lethals to 161 visibles, a ratio of 7.4 to 1. The somewhat lower ratio obtained in the experiment in this laboratory may be due to a difference in criteria of classification, or it may arise from an inherent difference in stocks. In any case the order of magnitude is the same.

Since the total mutation rate is composed of the sum of the mutation rates at the different loci, a comparative study of mutation at different loci is of interest. Obviously, sex-linked lethals are not adapted for such a study since there is no general means by which two such lethals can be tested for allelism, although Alikhanian[29] and Shapiro[30] were able to make such tests for loci that could be "covered" by the presence of a deleted X chromosome in addition to the undeleted X chromosome of lethal-bearing males. A comparative study of visible mutation rate could best be made by using an attached-X stock and examining individual males for new sex-linked visibles. However, it seems worth while to include the data that were obtained from over 70,000 test cultures examined. Although new sex-linked mutants have been found as individual males in cultures examined, these have not been tabulated, and all cases mentioned are of cultures in which all the Canton-Special males showed the visible. Each recurrence represents a new mutation at the locus; in the case of the goggle locus the two mutations recorded are probably a semilethal visible cluster. Of the total of 148 visibles and semilethal visibles found (these include some semilethal visibles at 25 r not recorded in Table 7.6), 47 were identified by their distinctive phenotypic appearance and by linkage tests as representing mutations at known loci in the X chromosome. Most of the others were probably also mutations of known loci, but in the absence of an easily identified and distinctive phenotype no attempt has been made to associate them with known loci. Thus many recessive minute bristle types have been found, but since this phenotype is found at several X-chromosome loci it is difficult to identify them. Table 7.7 presents the data on the mutants identified, giving the recurrences and the dosage at which each mutant appeared. It is to be remembered that at least some of the mutants occurring in the radiated material may be control mutations and not due to the radiation. No quantitative data for the white and Bar loci are given since these loci for technical reasons could not be investigated accurately in the genetic setup used. Several mutations to the Notch deficiency have been found but are not listed in the table. Table 7.7 includes a column showing the percentage of the total number of visibles that occurred at each locus and another column for comparison compiled from data on visibles in the report of Bridges and Brehme[9] based on over 600 visible mutations at sex-linked loci. (Bar and white loci were omitted in making this tabulation.) In general, the visible mutation picture in the two sets of data seems rather consistent. Visibles have been found recurring repeatedly at certain loci known from previous work to have a relatively high mutation rate. Possibly a more accurate comparison could be made if the percentages were doubled for each locus since about half the visibles included were semilethal visibles, which are not so likely to have been seen or, if seen, may have remained unanalyzed in previous work.

Table 7.7 — Record of Visible Mutations Found at Identified Loci

				Locus mutations (all visibles), %	
Visible	Locus	X-ray dosage for each recurrence, r	Total recurrences	This experiment	Complied from Bridges-Brehme[9]
Chlorotic	0.0	50; 4000	2	1.4	0.3
Yellow	0.0	500; 2000; 3000	3 (1)	2.7	8.6
Yellow achaete	0.0	50	1	0.7	0.6
Scute	0.0	4000	1	0.7	8.5
Rough eye	0.5	2000	1	0.7	0.2
Broad	0.6	150	1	0.7	1.1
Prune	0.8	150; 150	2	1.4	1.4
Oval	17.5	1000	1	0.7	0.2
Singed	21.0	4000; 4000; 4000	3	2.0	3.9
Goggle	25.0	150; 150	2	1.4	0.3
Lozenge	27.7	150; 500; 1000; 1000	4	2.7	3.1
Vermilion	33.0	Cont.; 500	2	1.4	3.1
Miniature	36.1	Cont.	1	0.7	3.9
Dusky	36.2	3000	1	0.7	1.6
Furrowed	38.3	Cont.; cont.; 500; 3000	4	2.7	1.4
Wavy	41.9	150	1	0.7	0.6
Garnet	44.4	500	1	0.7	3.3
Scalloped	51.5	3000; 4000	2	1.4	0.5
Uneven	54.4	1000	1	0.7	1.0
Rudimentary	54.5	Cont.; 150; 500; 1000; 1000; 2000; 3000	7	4.8	4.6
Forked	56.7	4000	1	0.7	5.2
Fused	59.5	150; 500; 1000; 4000	4	2.7	1.7
22 loci			47	31.8	55.7

It would appear that the recent mutation rates at the yellow, scute, miniature, garnet, and forked loci are lower than those reported previously but are higher at the furrowed rudimentary and fused loci. However, the numbers are too small to be significant. Of the 47 mutants listed in Table 7.7, 5 occurred in the controls. This is the same proportion of control visibles to radiation visibles as that found among all the visibles recorded in Table 7.6. On the basis of the relatively constant visible-lethal ratio at different dosages as indicated by data in Table 7.6, it may be inferred that approximately five more of the visibles listed in Table 7.7 from the radiation lots are actually control visibles. It would seem that most of the visibles listed in Table 7.7 were induced by the radiation. There is no way of knowing which of the specific visible mutations occurring in the radiation experiments were really spontaneous control visibles. Without this knowledge and with consideration of the relatively low number of visibles, it is premature to speculate whether or not relatively highly mutable loci, producing visibles even under control conditions, have a high induced visible mutation rate following irradiation. This is a problem worthy of future investigation. Extensive experiments on specific loci may show that some react differently from others in the relative numbers of control and induced visibles occurring. This, however, would be no proof that the total dosage–mutation curve for induced visibles differs from that for induced lethals. Loci that yield lethals may also react differently from others to radiation. The latter point would be more difficult to establish owing to the difficulty of identifying lethal loci. However, the problem might be attacked by studying lethal mutation rates at loci in which visible alleles occur. In this connection it is of interest to note that, in this study, among those lethals tested by linkage tests and not found to be associated with crossover inhibitors, 11 lethals were found so close to the scute locus as to give no crossing over among about 200 flies per test. Two such lethals were found near the crossveinless locus, three near the vermilion locus, eight near the forked locus, and none near the carnation locus. Thus, of 194 lethals not associated with crossover inhibitors and tested for linkage, 24 occurred in the regions of the five marker loci. Of the 148 visibles found, 11 were located at these five loci or gave no crossing over with them. If all semilethal visibles had been tested, it seems likely that several more visibles would have been found so close to these loci as to give no crossing over with them. The numbers are too small to establish any relation of mutation rate to dosage at the individual loci, but the data indicate that induced and control lethals and visibles both occur at specific loci. In the course of linkage tests, both visibles and lethals have been found associated with crossover inhibitors particularly abundant at high dosages. Although no cytological studies of these cases have been made, they probably represent gross chromosome rearrangements.

On the basis of the facts presented in this section the tentative conclusion was reached that the same relation of X-ray dosage to mutation rate holds for sex-linked visibles as for sex-linked lethals.

7. LINKAGE MAP AND CROSSOVER MODIFIERS

The linkage relations of 205 lethals and 39 visibles were studied by the method outlined in Sec. 2.1. In addition, the locus of a number of recurrent visibles was determined by crossing them directly to flies from stock that appeared to be the same visible or an allele. In the linkage tests 60,515 males were classified for the five marker genes used and, in the case of visibles, for the visible being located. This made an average of 248 males per linkage test. In only 3 cases were less than 100 flies classified, in 18 between 100 and 150 flies, in 27 between 150 and 200, and in all the rest over 200. In Fig. 7.4 the loci of 229 out of the 244 mutants studied are shown arranged in the order of their distribution on the genetic map of the X chromosome and in vertical columns representing their distribution in regions of five crossover units in length. Each locus has been assigned by calculation from the numbers of crossovers with the marker genes on either side of the mutant studied and by correcting the map distances thus determined by comparison with the standard map distance between the two marker genes involved. Whenever a mutant was found to lie in a region containing a significant crossover reducer, it has not been included in the figure. The greatest number of mutants was found at the extreme left end of the map, and the smallest number was found in the adjacent region 5 to 10. Other peaks occur in regions 20 to 25 and 55 to 60. Most of the mutants within the two highest peaks are found at or immediately adjacent to the scute and forked loci, respectively. On the whole the distribution of mutations along the length of the X chromosome which was found agrees well with the distribution described by earlier workers. Muller[14] was the first to provide such data. On the basis of extended observations he returned later to the same problem (Muller and Painter[31]). Similarly, Oliver[6] has studied the distribution of 233 lethals in a radiation experiment and finds peaks at or near the scute and forked loci. Slizynska and Slizynski[32] have investigated the distribution of 89 chemically induced sex-linked lethals from experiments of Auerbach and find a peak in region 0 to 5 and a low point in region 5 to 10. They state that the lowest frequency was found between 5 and 10 due to sudden drops around locus 10.

It is interesting to note in this connection that the longest blank region in the map of Fig. 7.4 is from region 9.4 to region 12.7, a map distance of 3.3 units. It should be kept in mind that the figures in the data presented here are based on the actual crossover counts and do not mean that there are as many as 172 loci in the X chromosome which can be separated by crossing over. Since some recurrent visibles have been mapped more than once and fall near but not at the same locus according to the linkage tests, chance variations of the same type have almost certainly occurred in the case of some lethals tested.

4.9												
4.9												
4.4												
4.3												
3.7												
3.5												
3.3												
2.6												
2.4												
2.2												
1.6												
1.5												
1.3											60.0	
1.2				24.7							59.8	
1.0				24.5							59.6	
0.9				24.4							59.6	
0.9				24.2							59.4	
0.9				24.1							59.2	
0.8				23.7			39.3			54.9	59.0	
0.8				23.7			39.5			54.8	58.4	
0.7				23.5			38.9			54.3	57.3	
0.5				23.4			38.8			53.9	57.1	
0.4				23.4	29.2		38.6			53.5	56.7	
0.4				23.3	29.1		38.4	44.5		53.3	56.7	
0.3				23.3	29.0		38.4	44.4		52.9	56.7	66.0
0.3				23.2	28.5	34.8	38.4	44.1		52.5	56.7	66.0
0.0			19.8	22.7	28.3	33.9	38.3	43.3		52.3	56.7	66.0
0.0			19.7	22.5	28.0	33.9	38.3	43.2	49.8	52.1	56.7	66.0
0.0		14.8	19.2	22.5	27.6	33.5	38.2	42.8	48.8	52.0	56.7	65.2
0.0	9.4	14.8	19.2	22.4	27.4	33.0	38.1	42.8	48.7	51.7	56.7	64.8
0.0	8.0	14.6	18.8	21.5	27.3	33.0	37.8	42.8	48.6	51.3	56.7	64.1
0.0	6.1	14.1	18.8	21.4	27.3	33.0	37.0	42.4	48.5	51.1	56.6	63.9
0.0	6.0	14.1	18.7	21.2	27.2	32.3	36.6	42.2	47.1	50.8	56.4	63.9
0.0	5.9	13.7	18.7	20.8	26.7	32.1	36.6	41.5	46.8	50.8	55.5	63.5
0.0	5.8	13.7	17.7	20.8	26.2	32.1	36.6	41.3	46.2	50.4	55.4	63.0
0.0	5.7	13.1	16.4	20.7	26.1	30.6	36.2	40.9	45.7	50.4	55.4	62.5
0.0	5.3	13.0	15.8	20.4	26.0	30.4	35.9	40.7	45.6	50.3	55.3	60.8
0.0	5.1	12.7	15.4	20.2	25.3	30.2	35.2	40.1	45.1	50.1	55.2	60.8

Fig. 7.4 —Graphic record of locus determinations of 229 lethals and visibles from control and radiation experiments, showing high- and low-mutation-rate regions along the genetic map of the X chromosome (see Sec. 7 for full explanation and comparison with data of other workers).

During the course of the linkage tests it was noted that crossing over seemed high in the left end of the chromosome, the region from scute to vermilion. A separate linkage test was therefore run, involving 30 cultures of Canton-Special/sc-cv-v-f-car females to

Muller-5 males. Tabulation of the 2790 males gave 15.7 per cent crossovers between sc and cv, 23.4 per cent between cv and v, 26.6 per cent between v and f, and 5.7 per cent between f and car. The standard map distances (Bridges and Brehme[9]) for these regions are sc-cv, 13.7; cv-v, 19.3; v-f, 23.7; and f-car, 5.8. It will be noted that the left end of the X chromosome gives relatively high crossover values.

Using the map derived from this experiment instead of the standard map, the crossover values for each of the four map regions in each of the 244 linkage tests of lethals and visibles were examined for significant deviations from the values given above. Significantly high crossover values were found repeatedly in the two regions to the left of vermilion, a portion of the X chromosome which has generally been found relatively free from variations in crossing over (see Bridges,[33] Plough,[34] and Kikkawa[35]). However, no attempt was made to analyze these cases further, and it is not known whether they were due to genetic or environmental modifiers or both.

Table 7.8 — Crossover Suppressors Associated with Lethals and Visibles at Different X-ray Dosages*

Dosage, r	Lethals tested	Crossover suppressors (regions 1,2,3,4)	Visibles tested	Crossover suppressors (regions 1,2,3,4)
Control	32	0	5	0
25	4	0	0	0
50	21	1,2,3,4	2	0
150	48	3,4	7	0
500	8	0	4	0
1000	77	1,2,3; 1,2,3; 1,2,3,4	8	3,4; 1,2,3,4
2000	3	0	3	0
3000	9	3; 4; 1,2,3 1,2,3,4; 1,2,3,4	7	2; 3
4000	0	0	6	1; 1
Total	202	10	42	6

*sc-cv, region 1; cv-v, region 2; v-f, region 3; f-car, region 4. 1,2,3,4 indicates crossing over suppressed, or nearly so, throughout the chromosome from scute to carnation.

Of more interest in connection with this study were a number of crossover suppressors that eliminated or very strongly reduced crossing over. Table 7.8 presents the data. It should be stated that linkage studies were undertaken only to demonstrate that the lethals and visibles obtained were actually different from one another and not to study crossover suppressors. Most of this work was done during the first year, and few lethals at dosages above 1000 r have been analyzed for linkage relations. However, it will be noted that, of the 16 crossover suppressors reported, 9 were associated with lethals or visibles at 2000 r or above. Only 28 mutants at these dosages were studied for linkage relations. Five more of the crossover suppressors were found among 85 lethals and visibles from the 1000-r lots and only two from the 131 lethals and visibles at dosages of 500 r or less. Lea and Catcheside,[36] after reviewing the evidence from data of Timoféeff-Ressovsky,[37] Oliver,[6] Demerec,[38] and Demerec and Fano,[39] came to the conclusion that gross chromosome rearrangements, which have been shown to increase approximately as the $\frac{3}{2}$ power of the dosage (see Muller[40]), do not in themselves constitute a separate class of lethals but rather that lethals, of which a large class occurs at chromosome breakage points, are therefore often associated with gross rearrangements at high dosages. The data in Table 7.8 indicate that visibles also tend to be associated with gross chromosome rearrangements at high dosages. Very extensive data of Patterson, Stone, Bedichek, and Suche[41] on reciprocal translocations in Drosophila indicate that over two-thirds of 466 cases studied were not viable in homozygous condition under the culture conditions employed by these workers and were therefore associated with or were recessive lethals or semilethals. The presence of a large group of chromosome rearrangements after high X-ray dosages makes it difficult to determine actual mutation rates because of the chance of two or more mutations being associated with such a rearrangement and therefore not easily separable. For a study of the relation of dosage to mutation, however, this problem becomes less serious at dosages on the order of 1000 r.

8. TECHNICAL PROBLEMS OF MUTATION-RATE STUDIES

Since large-scale mutation-rate experiments on control material, low dosages of X rays, and other extrinsic agents that induce mutations may subsequently be undertaken by others, it seems pertinent to discuss some of the technical problems which are involved in this type of work.

In the first place much attention should be given to the design of the experiment. The planning of a large-scale experiment should take into consideration the testing of one or more reasonable hypotheses with particular attention paid to a proper balance between uniformity of procedure in collection of data and a certain degree of flexibility as the experimental data are accumulated. Careful consideration should be given to the ratio between control and experimental data.

These data should, in so far as possible, be collected in such a way that unknown variables are most likely to be distributed at random to control and experimental material. This involves a careful distribution of control data in time as well as in space. The advice of a trained biometrician, who not only understands statistical procedure but is conversant with the technical difficulties involved in the specific program, is invaluable both before the initiation of the experiments and from time to time during the period when data are being collected. A few hours of careful planning may save months of laborious work and give a maximum yield of valid data for the time and effort expended.

Since experiments of this type will extend over a relatively long time period, special attention should be given to establishing a routine whereby the work will be done on a uniform schedule. Small changes in laboratory technique which eliminate unnecessary labor become highly important. Every unnecessary step eliminated makes possible the collection of that many more data and consequently increases their reliability.

Careful attention should be given to the choice of personnel for experiments of this kind. A large proportion of the work is of a routine nature; yet the individuals doing this work must be extremely careful and accurate and have an honest appreciation of the consequences of errors that might invalidate the data. That part of the procedure where such errors are most likely to be made should be handled by the members of the personnel who thoroughly understand the necessity of avoiding these errors.

Careful consideration should be given to the choice of the biological material used in mutation-rate studies. The genetic tools developed primarily by Muller make possible mutation-rate studies on Drosophila melanogaster on a scale and at a level not at present attainable in any other animal. The Muller-5 stock has proved almost ideal as a tool for the study of lethal mutation rates in the X chromosome of this species. Not only can sex-linked lethals be rapidly and accurately detected, but, after they are found, they can easily be maintained in stock by the use of this tool. The short life cycle, ease of culture, and high fecundity of this species make it excellent material for mutation-rate studies. Data from this species must long serve as a base of reference for work on other organisms.

Eventually the natural and low-radiation mutation-rate studies on specific genetic loci may be undertaken. First, however, the general mutation rate in an entire chromosome under different conditions needs to be established. Reasons have been given for the choice of pure lethals as the category of mutations to be studied. However, data from both visibles and lethals indicate variable mutation rates at different loci. Yet the general mutation picture for visibles and lethals under low radiation appears comparable (see Table 7.6). Therefore, since the total control and low-radiation mutation rates for the entire X chromosome are so low that they constitute a limiting factor in the collection of valid data, any procedure that will allow the inclusion of mutational categories other than pure lethals on the same objective level and with little additional work seems desirable.

In these experiments the Canton-Special stocks, from which the chromosomes being tested were derived, were kept at constant temperature, 24 ± 1°C, whereas the experimental test cultures reared for the detection of lethals were kept on open shelves in cabinets in the laboratory, where rather wide fluctuations in temperature occurred at different seasons of the year. This procedure resulted in some lots of experimental cultures being reared under temperature conditions where the emergence of the flies in the cultures was further advanced at the time the cultures were examined than in other lots. Furthermore certain mutants behave as semilethals at one temperature and as lethals at another temperature (see Dobzhansky and Spassky[20]). Many examples of mutants that behaved as lethals in the first generation and as semilethals in the second generation were found. Some which appeared as lethals in the two test generations acted as semilethals in later linkage tests. As explained above, all mutants that were scored as pure lethals in the first generation were included in calculations of mutation rate. However, if the cultures had been reared at constant temperature, a rather large group of semilethals and delayed-emergence mutants with visible effects, discovered through the total absence of the semilethal or delayed-emergence class when the cultures were first examined, could have been included in the totals. The experience with such mutants would indicate that the inclusion of these classes would increase by 10 per cent or more the total mutations discovered, a considerable figure when low mutation rates constitute a limiting factor in these studies. In any case, test cultures should be reared at constant temperature to cut to a minimum inaccuracy in objective scoring of that class of mutants which is semilethal at one temperature and lethal at another.

The experience with lethal clusters in this study emphasizes the importance of developing sampling methods that will reduce errors from this source without being unreasonably cumbersome and time-consuming. Obviously a compromise solution is indicated.

Finally, every reasonable precaution should be taken to measure accurately the X-ray dosages administered. To the biologist working in this field, fluctuations in the output of X-ray machines and in the performance of dosimeters used in checking this output constitute an exasperating source of error that must be compensated for in so far as possible by the design of the experiment. The chances are much better that the radiations received actually bear the relation to one another that the readings indicate if many small lots of flies are irradiated at weekly intervals and if the radiation experiments at different dosages are run in parallel than if all work at one dosage is done at one time and that at another dosage at another time. Even with the best physical equipment available the chance of errors from faulty measurement of dosage should be realized, and every precaution should be taken to keep them at a minimum. No attempt was made in these experiments to apply the radiation at constant temperature. The laboratory in which the radiation was carried out was not temperature controlled. With the accumulation of evidence that mutation rate may be influenced by secondary extrinsic factors, it would seem that some attention might well be given to temperature control during radiation. It is not believed that this factor introduced any large error in the data presented here, yet it might well be controlled in future experiments in the interest of eliminating a possible variable.

9. SUMMARY

Using a tool stock supplied by H. J. Muller, 200,801 X chromosomes of mature sperm of a Canton-Special stock of Drosophila melanogaster were tested for the origin of new lethal mutations.

Of these chromosomes, 73,901 were controls, 51,907 were subjected to X rays at a dosage of 25 r, 31,560 at 50 r, 23,195 at 150 r, 6,634 at 500 r, 6,977 at 1000 r, and smaller numbers at 2000, 3000, and 4000 r.

From these tests it is concluded that, even for X-ray dosages as low as 50 and 25 r, the linear relation of dose to mutation frequency holds.

From all the data collected, on the basis of this linear relation, the control lethal mutation rate in the Canton-Special stock tested was calculated as 0.1004 per cent and the induced mutation rate per roentgen as 0.00215 per cent. When only data at dosages of 1000 r and lower were included, the control mutation rate was calculated as 0.1039 per cent and the induced mutation rate per roentgen as 0.00197 per cent.

These data indicate that the control mutation rate was doubled at an exposure to about 50 r. The apparent discrepancy between this figure and that of 35 r derived from earlier X-ray experiments may be explained on the ground that sperm cells of young males, which have been shown by Muller to give a high control rate, were used exclusively in this experiment.

Several cases of control lethal clusters were found and are interpreted as due to mutations occurring in early stages in the germ tract. The problem of sampling sperm cells is discussed in this connection.

The implications of the findings in connection with the exposure of large numbers of human beings to low dosages of X rays are discussed briefly.

A study of visible mutation rates, reported in Sec. 6, has given an over-all lethal-visible mutation ratio of 5.2 to 1.

The data on visibles are not inconsistent with the view that the linear relation of dosage to mutation holds at low dosages for this class of mutations as well as for lethals.

In Sec. 7, linkage studies on 229 lethals and visibles are reported. Relatively high mutation rates for both lethals and visibles were found in genetic chromosome map regions 0 to 5, 20 to 25, and 55 to 60, and a relatively low rate near 10.

Mutations in control chromosomes and in those subjected to low dosages of X rays were rarely associated with crossover suppressors, whereas mutations in chromosomes receiving high radiation were frequently associated with crossover suppressors.

A discussion of technical problems in connection with large-scale mutation-rate studies at low dosage is included in Sec. 8.

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Chapter 8*

COMPARISON OF X- AND BETA-RADIATION EFFECTS IN RABBITS

By Francis W. Bishop

Prior to the time of the development of the atomic bomb there were relatively few reports in the literature on the effects of pure beta rays on biological material. No really high-intensity source of beta rays was available, and radioactive material from these rays had not become an industrial hazard.

Baker[1] in 1934 made a quantitative comparison of the effects of the beta rays of radium on the agent of Rous sarcoma, on bacteriophage, on tetanus toxin, and on certain bacteria, antibodies, and enzymes. Spencer[2] in 1935 reported effects of beta rays from radium on bacteria.

Radium plaques have been used to some extent in the treatment of superficial lesions in man, but relatively little work has been done to compare beta-ray and X-ray effects on appropriate biological subjects. It is to be expected that, on thick biological material, equal quantities of X rays and beta rays might produce different effects since the beta rays are absorbed in the relatively thin surface layer, whereas the X rays may penetrate the entire thickness of the animal.

Raper, Zirkle, and Barnes[3] compared the effects of beta and X rays on numerous biological objects, including fern spores and Drosophila eggs. They applied total-body radiation from intense beta-ray sources to mice, rats, guinea pigs, and rabbits and also treated restricted areas of rabbit and human skin. They found that, in the case of the fern spores, the median lethal dose (LD_{50}) for inhibition of cellular division, by beta rays, was approximately 63,000 rep and, by 200-kv X rays, about 58,000 r. The dose of beta rays required for 50 per cent killing of Drosophila eggs was 270 rep, and the dose of gamma rays from Ta^{182} for the same result was 270 r.

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* Based on Report MDDC-203 (II-188-5935).

Russ[4] in 1923 compared the effects of hard and soft X rays on animal tissues and concluded that, for an equal dose of each, the appearance time of the reaction was later in the case of the hard rays and that larger dosages of hard rays could be tolerated.

Packard[5] found that X-ray beams of equal intensity but of different wavelengths produced the same quantitative biological effects on Drosophila eggs. The LD_{50} was quoted as 180 r with wavelengths varying from 0.20 to 0.70 A, corresponding to intensities ranging from 95 to 30 kv. This range was later extended to include 12- and 550-kv X rays.[6]

The object of the experiments reported in this chapter was to determine the effects of comparatively low-voltage X rays on the skin of a suitable animal and, if possible, to compare these effects with those obtained by exposing similar skin areas to sources of beta rays.

An X-ray machine was assembled in the laboratory that was capable of delivering approximately 25 r/min at 30 kv, 5 ma, with no filtration other than the thin glass wall of the tube and with a target-skin distance (TSD) of 8 in. The X-ray intensity was measured with a Victoreen r-meter using a 25-r thimble chamber.

A number of ½-in.-diameter disks of ¼-in.-thick phenolic formaldehyde resin, impregnated with amorphous red phosphorus and activated in the Clinton pile, were used as a source of beta rays. These sources were calculated to have an activity corresponding to 145 rep/min at the time that they were shipped to the laboratory, and the subsequent activity was determined from the characteristic decay curve. This material is described by Raper, Zirkle, and Barnes.[3]

Twenty-eight rabbits were treated over 167 areas with unfiltered 30-kv X rays with dosages ranging from 600 to 2000 r. Fourteen rabbits were treated on 68 corresponding areas with the beta-ray source and with dosages ranging from 1500 to 10,000 rep. The active disks were countersunk and cemented into protective blocks of lucite and fastened to the ear of the rabbit with adhesive tape. The animals were under observation for periods of from 30 to over 200 days; the experiments were interrupted in some instances by the death of the animal from extraneous causes.

1. X RAYS

Sixteen of the animals were exposed to X rays on the inner aspect of the ear, and the remainder were exposed on the external surface.

There was no marked difference in the character of the response except that slight degrees of epilation were more easily seen on the external or more hairy surface of the rabbit ear.

The first erythemata were observed in animals receiving 1000 r. Of these, 16 per cent showed mild erythema about 30 days after treatment. Slight epilation without erythema had been noted in one animal which had received 900 r.

At dosage levels of from 1000 to 1400 r a large proportion of the animals were permanently epilated, and these animals developed from very mild to moderate erythema without sequelae. Most of the animals that received from 1400 to 2000 r showed marked erythema, often with vesiculation followed by eschar and, finally, in some of the animals under observation for several months, by cutaneous atrophy. All these animals were permanently epilated. At any particular dosage level which produced both epilation and erythema, the appearance of the erythema generally preceded that of the epilation by about 3 to 5 days. It may be noted that the lowest dosage which brought about epilation in 100 per cent of the areas treated was considerably less than the lowest dose necessary to produce an erythema in 100 per cent of the cases. From Table 8.1 it may be seen that all animals receiving 1400 r or more were permanently epilated, whereas it required a dose of 2000 r to produce an erythema in 100 per cent of the same areas. With dosages of 1100 r and above, many animals showed increasing degrees of epilation on the exit side of the ear. This was observed in about 4 per cent of the areas receiving 1100 r and in 100 per cent of the areas receiving 2000 r. Absorption measurements showed that approximately 25 per cent of the radiation was absorbed in the ear. The average thickness of the rabbit ear varied from 0.75 mm near the tip to 1.37 mm near the base. Scaliness of the skin was a common reaction at epilating dosages and above. The data on 167 treatments with 30-kv X rays are summarized in Table 8.1.

2. BETA RAYS

Preliminary tests indicated that the threshold erythema might be expected in the neighborhood of 2000 rep with the beta rays from P^{32}. Accordingly, a group of rabbits were exposed to dosages of from 1500 to 10,000 rep from the active disks on the inner surface of the ear.

The first erythematous reactions were noticed in a series of 10 areas exposed to 3000 rep of beta radiation, 40 per cent of the animals treated developing a slight reddening about 18 days after treatment. Some scaliness of the skin was noted in 18 per cent of the animals receiving 2000 rep, although no other reaction occurred. As in the

Table 8.1 — Effects of 30-kv X Radiation on the Rabbit Ear

		Scaliness			Epilation			Erythema		
Dose, r	Number of areas	Appearance time, days	Duration, days	% showing reaction	Appearance time, days	% showing reaction	% epilation (opposite side ear)	Appearance time, days	Duration, days	% showing reaction
2000	2	?	?	100	?	100	100	?	?	100
1800	13	17	104	92	21	100	77	18	27	77
1600	13	19	87	85	22	100	77	22	14	77
1400	21	28	36	66	32	100	0	28	26	90
1300	27	37	29	21	34	74	38	30	29	93
1200	38	28	48	42	29	82	26	29	18	87
1100	28	43	13	36	37	82	4	34	13	61
1000	18	39	?	39	35	72	0	31	18	17
900	3	0	0	0	41	33	0	0	0	0
800	2	0	0	0	0	0	0	0	0	0
600	2	0	0	0	0	0	0	0	0	0

case of the X-rayed animals, the appearance of an erythematous reaction preceded the onset of epilation. No instruments were available in this laboratory for the measurement of absorption of the beta rays in the rabbit ear, but with dosages of 8000 rep and higher an increasing number of the areas treated began to show epilation on the opposite side of the ear. The erythematous reactions were usually mild to moderate up to 4000 rep, but one rabbit developed strong erythema and vesiculation 25 days after 3500 rep.

All animals receiving 6000 rep and above were permanently epilated, and almost 100 per cent of them developed an erythema at levels of 7000 rep and above. The percentage epilated and showing erythema was less (but fairly high) down to dosages of 3000 rep. The data are compiled in Table 8.2.

The results from the use of the plaques are considerably more erratic than in the case of the X-ray experiments, possibly owing to the difficulty in maintaining accurate and intimate contact with the skin in the presence of various amounts of hair and cutaneous folds.

In a few instances, histological sections were made of the rabbit ear at the height of reaction and following the abatement of symptoms.

One rabbit receiving X rays was examined at the height of the reaction (33 days posttreatment) at dosages of 1000, 1100, 1200, and 1300 r. Another X-rayed animal which had received 1200, 1400, 1600, and 1800 r was examined 150 days posttreatment.

In the beta-ray group one animal which had received 3500, 4000, 4500, and 5000 rep was examined 57 days after treatment, at which time the acute reaction had subsided. The ear from a second animal which had received 2000, 3000, 4000, and 5000 rep was sectioned 91 days posttreatment. A third ear which had received 5000, 6000, 7000, 8000, 9000, and 10,000 rep was sectioned 119 days posttreatment.

The following pathological report was received from Roger Metcalf and Frank Inda, who studied the histological material: In the sections made 33 days postradiation, the area receiving 1000 r showed no histological change from the normal. In the 1100-r specimen there was scant lymphocytic infiltration. Hair follicles were few in number. At 1200 r, slight but definite connective-tissue edema was noted. Mild eosinophil and round-cell infiltrations were present. Local loss of surface epithelium and adjacent hemorrhage and epithelial thickening coincided with the presence of black pigment, suggesting trauma from tattooing of the skin. There was moderate capillary increase with congestion. At 1300 r, edema of a mild degree was again present in the connective tissue with the presence of eosinophil and lymphocytic infiltrations. Hair follicles were absent on the internal surface of the ear.

Table 8.2 — Effects of Beta Radiation on the Rabbit Ear

		Scaliness			Epilation			Erythema		
Dose, rep	Number of areas	Appearance time, days	Duration, days	% showing reaction	Appearance time, days	% showing reaction	% epilation (opposite side ear)	Appearance time, days	Duration, days	% showing reaction
10,000	3	36	21	100	44	100	100	36	9	100
9,000	3	31	17	100	44	100	100	28	13	66
8,000	3	45	?	66	44	100	33	26	19	100
7,000	3	33	30	100	44	100	0	37	42	100
6,000	3	32	26	33	44	100	0	28	42	33
5,000	13	24	19	77	23	46	0	18	16	61
4,000	10	22	20	100	?	20	0	20	12	100
3,500	4	20	42	50	48	25	0	18	12	50
3,000	10	25	13	60	0	0	0	18	15	40
2,500	4	24	25	50	0	0	0	0	0	0
2,000	11	23	4	18	0	0	0	0	0	0
1,500	1	0	0	0	0	0	0	0	0	0

In the sections made 150 days after X irradiation, those at the 1200-r level showed only slight epithelial thickening. There were no other significant changes. At 1400 r the hair follicles were fewer in number but otherwise normal. No noteworthy changes in the connective tissue or epithelium were encountered. At 1600 r the hair follicles were atrophic, and an occasional small local hemorrhage was found. At 1800 r the hair follicles were scant and atrophic. The connective tissue was arranged in parallel fibers in contrast to the haphazard arrangement normally seen.

In the group receiving beta irradiation, sections made 57 days post-treatment gave histological appearances as follows:

Radiation, rep	Changes
3,500	Changes too subtle to be significant
4,000	A few atrophic hair follicles; connective tissue and epithelium histologically normal
4,500	Connective tissue arranged in parallel strands; hair follicles few in number on external surface of ear; those remaining were atrophic
5,000	No section

The following changes were noted in the sections made 91 days post-treatment:

Radiation, rep	Changes
2,000	No significant findings
3,000	Hair follicles normal; connective tissue normal
4,000	A few atrophic hair follicles were noted; moderate atrophy of epithelium present; connective tissue normal
5,000	Small area of superficial connective-tissue necrosis covered by thin layer of reepithelization; hair follicles atrophic; connective tissue showed sparse pycnotic nuclei; small local area of hemorrhage

The following changes were noted in the sections made 119 days post-treatment:

Radiation, rep	Changes
5,000	A few atrophic hair follicles present near the external surface; a mild infiltration of eosino-

	phils and lymphocytes; an occasional small interstitial hemorrhage
6,000	A few atrophic hair follicles present near the external surface of the ear; local atrophy of the epiderm was noted
7,000	Isolated epithelial inclusions present in the connective tissue beneath the epiderm; in addition, the surface epithelium was atrophic
8,000	Atrophic hair follicles noted near the external surface of the ear; connective tissue again arranged in parallel strands; connective-tissue nuclei, sparse and pycnotic
9,000	Hair follicles absent on external surface; sebaceous glands difficult to find; a few small local hemorrhages identified
10,000	An occasional atrophic hair follicle noted near the external surface; connective tissue again arranged in parallel strands; a few small local collections of lymphocytes identified

3. DISCUSSION

The most significant changes were observed in the sections made 33 days after X irradiation. Edema of the connective tissue, vascularity, and inflammatory cell infiltration stamped the ears as having definite injury, presumably a radiation change. The absence of hair follicles on the internal surface of the ear was not significant since the normal rabbit ear also shows lack of these hair follicles in a similar position. The local epithelial loss with associated hemorrhage was interpreted as being due to tattooing since the changes were immediately adjacent to heavy black pigment deposits in the superficial tissue. No damage was apparent in the cartilage.

In the group in which 150 days had elapsed since exposure, no significant changes were noted except in the 1600- and 1800-r specimens. Even in these specimens the changes were not marked but were evidenced by scant numbers of atrophic follicles and the parallel arrangement of the connective-tissue strands, the latter in contrast to the haphazard arrangement normally seen.

In the beta irradiation, those ears examined 57 days after exposure showed changes in the 4500-rep specimen evidenced by the parallel arrangement of the connective-tissue strands and a few atrophic hair follicles near the external surface of the ear. The 3500- and 4000-rep specimens failed to show conclusive evidence of irradiation effect. No section of the 5000-rep exposure was present.

In the sections made 91 days after treatment, only the 4000- and 5000-rep specimens showed changes. In the latter exposure an area of connective-tissue necrosis with reepithelization was still present 91 days after treatment.

In the sections examined 119 days after treatment, all exposures from 5000 to 10,000 rep showed changes. In the lower exposures the changes were less marked but became more apparent toward the higher exposures.

It should be emphasized that the changes described are not dramatic; they are difficult to evaluate.

4. CONCLUSIONS

From Table 8.1 it can be seen that the minimal dosage necessary to produce an erythematous reaction in the ear of the rabbit with 30-kv X rays is in the neighborhood of 1000 r. A somewhat smaller dose (900 r) was able to cause epilation without erythema. Generally the phenomenon of epilation appears at a lower dosage level, or a higher percentage of the animals are epilated than show erythema for a given dosage. As might be expected, the erythema appears sooner and generally lasts longer at the higher dosage levels, and epilation also occurs earlier under these circumstances. Scaliness of the areas treated was a usual accompaniment of the erythema, but its appearance time and duration were erratic.

Table 8.2 indicates that the lowest dose of beta rays capable of causing an erythema in the rabbit ear is near the level of 3000 rep. Epilation was not observed until a 3500-rep dose had been administered, and the percentage epilated was less for a particular dose than that developing erythema. Generally the appearance time of the epilation was considerably longer than that of the erythema. Scaliness without any other sequelae was noted at low dosage levels but was as variable as in the case of the X-rayed rabbits.

These few experiments indicate that from 3 to 5 times the dosage is necessary in the case of beta rays to produce a given effect, e.g., it required approximately 3 times the dose of beta rays as compared with low-voltage X rays to produce a minimal erythema, about 4 times the dose to cause the first signs of epilation, and about 4½ times the dose to produce epilation in all the animals treated.

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PART II

CHRONIC X RADIATION

Chapter 9*

OBSERVATIONS ON ANIMALS EXPOSED TO WHOLE-BODY X RADIATION IN DIVIDED DOSES OVER LONG PERIODS: INTRODUCTION AND TECHNIQUES

By Andrew H. Dowdy, Robert D. Boche, and Francis W. Bishop

These experiments were undertaken to determine whether the accepted tolerance levels for exposure of persons to X and gamma radiation are in fact adequate to ensure a reasonable degree of safety. The history of the tolerance dose has been reviewed by Henshaw,[1] and it is clear from his paper that the setting[2] of 0.1 r/day as the amount of exposure which persons may sustain without detectable injury is, at best, only an intelligent estimate. It should be noted that earlier[3] and later[4] recommendations have been in favor of a tolerance dose one-fifth that currently accepted in the United States; the standard in England is said to be 0.2 r/day.

The general objectives of these experiments were (1) to determine whether there exists any exposure level to which animals may be subjected without the manifestation of any deleterious effects and, if so, to determine this level of radiation exposure and (2) to find which organ system is the most sensitive to chronic radiation injury and to collect further data on the nature of this hazard. This work occupied about 2½ years and involved approximately 50 people, some full time. More than 11,000 X-ray exposures were given to approximately 400 rats, 100 dogs, 100 monkeys, and 100 rabbits.

In addition, a large-scale parallel experiment was conducted by Donald R. Charles on the influence of chronic X radiation on mice, with particular emphasis on changes in genetic constitution and related effects. The results of these experiments are summarized elsewhere.[5]

*Based on Report MDDC-254 (II-188-5979).

Two sources of radiation were used: a 1000-kv General Electric industrial unit and a Picker 250-kv industrial unit. The animals were equally divided according to which of these sources furnished the radiation that they received, and the exposures were given 6 days per week. Table 9.1 shows the structure of the experiment.

Table 9.1 — Structure of Experiment

Dosage rate, r/day	Number of animals			
	Rats	Dogs	Rabbits	Monkeys
1000-kv X Radiation; HVL,* 5.12 mm Pb				
0.1	50	10	10	10
0.5	50	10	10	10
1.0	50	10	10	10
3.0		5		
6.0		5		
10.0	25	10	10	5
250-kv X Radiation; HVL, 2.1 mm Cu				
0.1	50	10	10	10
0.5	50	10	10	10
1.0	50	10	10	10
3.0		5		
6.0		5		
10.0	25	10	10	5
Controls	50	9	16	5

*Half-value layer.

Not all dog experiments were run concurrently; the 10 r/day dogs were treated in four groups of five animals; two experiments were run concurrently and two consecutively. The original group of five controls was increased to nine during the course of the experiment. The last four that were added served for general survival, sperm counts, etc., but were not studied otherwise.

Animals were irradiated until death or termination of the experiment, at which time most of them were autopsied. A 24-hr surveillance of the animal colony, including Sundays and holidays, generally ensured autopsy within 1 hr after death.

1. EXPERIMENTAL ANIMALS

1.1 Rats. Irradiation of rats was commenced Dec. 6, 1943, at which time they were all between 3 and 4 months of age. Approximately equal numbers of males and females were used in each group. Initial blood cell counts and body weights were obtained before the start of irradiation, and subsequent measurements and physical examinations of each animal were made at regular intervals as shown in Table 9.2.

The rats were grown in the laboratory from stock which originated from the Wistar strain some years before. They were housed two to a cage, the sexes separate. They were fed Purina Fox Chow (with meat meal) and water ad lib., and on Saturdays they received a supplement of Fox Food Blox (Allied Mills, Inc.) to sustain them over the week end. They were coded by groups by amputation of one or two toes and by animal number within groups by ear punching. Each cage was scrubbed with a brush and hot water and steamed once each week. At the end of 2 years of radiation, approximately one-third of the surviving animals were randomly sacrificed and studied for pathological changes. The remainder were given a 3-month rest period, during which time routine blood and other studies were continued for the purpose of analyzing recovery from presumptive damage. At the end of this rest period, all surviving rats were sacrificed and studied for tissue changes.

Table 9.2—Schedule for Examinations and Measurements

	Controls	0.1 r/day	0.5 r/day	1.0 r/day	3.0 r/day	6.0 r/day	10.0 r/day
			Interval Between Blood Studies, Weeks				
Rats	8	8	8	8			2
Dogs	4	8	8	8	1	1	1
Rabbits	4	8	8	4			1
Monkeys	4	8	8	8			1
			Interval Between Weight and Clinical Examinations, Weeks				
Rats	1	4	4	4			1
Dogs	1	4	4	4	1	1	1
Rabbits	1	4	4	4			1
Monkeys	1	4	4	4			1

1.2 Dogs. A large proportion of the dogs were young but fully grown beagles, although some mongrels had to be used. They were given veterinary examinations for parasitic round and flat worms and other defects initially and periodically during the course of the experiment and were immunized against distemper before use. The dogs were housed separately on newspaper beds; their cages were washed and steamed once daily. Bedding was changed daily or oftener if necessary. All dogs were fed Friskies (Albers Milling Co.) during the first 19 months of treatment and a diet of 1 part Eveready dried meat meal to 4 parts moist Friskie meal during the last 5 months of irradiation. Food was given in the afternoon daily except Sunday, and water was available at all times. They were treated occasionally for mange, and this showed no relation to radiation. Internal medication, except for worms, was avoided to prevent complications in interpreting the data on blood and other variables. Dogs were examined, weighed, and bled for hematological and biochemical study initially and at regular intervals (see Table 9.2).

Sperm counts were first made on male dogs 9 months after the start of the experiment and at irregular intervals thereafter. Irradiation was begun Jan. 10, 1944, and continued until death or until the number of treatments listed in Table 9.3 had been accumulated.

Table 9.3 — Radiation Test Data

Daily dose, r	0-I	0-II*	0.1	0.5	1.0	3.0	6.0	10.0
Number of treatments	622	500*	622	622	622	500	500	500
Time, months	24	19.2	24	24	24	19.2	19.2	19.2

*Added to experiment with 3.0- and 6.0-r groups.

Control dogs were maintained in their kennels and were not transported through the halls to the treatment rooms. Controls in all the other species were handled and transported the same as the experimental animals but were not X-rayed. A treatment for a control rat, rabbit, or monkey consists, therefore, in much handling and transportation, whereas, for a control dog, treatment was merely a day of survival on which experimental dogs were treated. At the conclusion of 500 treatments the surviving dogs on the 3, 6, and 10 r/day schedules were sacrificed and studied by the pathology division. A random sample of one-third of the surviving dogs in the groups receiving 0, 0.1, 0.5, and 1.0 r/day was sacrificed at the conclusion of 2 years or 622 treatments. The remaining females were given a period of 3 months for blood-recovery studies and then were sacrificed. The surviving males that were not sacrificed were retained for studies on recovery in sperm count.

1.3 Rabbits. The rabbits were of varied origin, were caged separately, and were fed on a diet composed of equal parts of oats and Purina Rabbit Chow Pellets. They were given food and water ad lib. and were weighed and bled according to the schedule presented in Table 9.2.

X radiation of these animals was begun in December 1944 and was continued for 1 year or 310 treatments. At the end of this period one-third of the surviving rabbits were autopsied, and their tissues were studied histologically. The remaining survivors were given a 3-month recovery period as with rats and dogs.

1.4 Monkeys (Macaca rhesus). Procedures with monkeys paralleled those with other species. Chronic irradiation was begun in December 1943 at the daily doses shown in Table 9.1. The very high incidence of tuberculosis in the colony, which was the major cause of death, tended to obscure effects of radiation from nearly every standpoint. However, some information was obtained and is included to complete the report of the experiments. Unlike other species the monkeys were housed together, usually five to a cage, with the sexes together. Considerable care was taken with the sanitation, which included steaming the cages daily. Their diet was varied and included fresh vegetables, bread, oranges, and milk as staples.

Because of the high mortality, continuous replacement of the monkeys during the first 4 months of treatment was practiced, i.e., when a monkey died, it was immediately replaced by another monkey which was then treated as an additional animal. This merely complicated the protocols and meant that irradiation at the end of a 2-year period could not be stopped simultaneously on all monkeys. As with other species, a proportion of the survivors were sacrificed at the end of 2 years; the remainder were permitted a period in which to recover from possible effects and were then sacrificed. Like the other species they were weighed and bled according to the schedule in Table 9.2.

At the dosage levels used in these experiments, slight decreases in average values for a variable such as white blood cell count could not always be shown to be statistically significant. Additional indications in regard to the reality of an effect could be obtained if, during the rest period, some evidence of a corresponding increase or recovery was observed. In the case of effects on sperm counts, this period provided crucial information at the lowest dosage levels. During the recovery periods, therefore, animals were transported to the treatment rooms as usual, permitted to remain there as if being radiated, and then returned to their colonies.

2. RADIOLOGICAL TECHNIQUE

The schedules were so arranged that rats and dogs were irradiated in the morning and monkeys and rabbits in the afternoon. Geometrical arrangements and the relation between the 250- and 1000-kv machines and the animal cages are shown in Fig. 9.1. Figure 9.2 shows the circular arrangement of the treatment cages as seen from

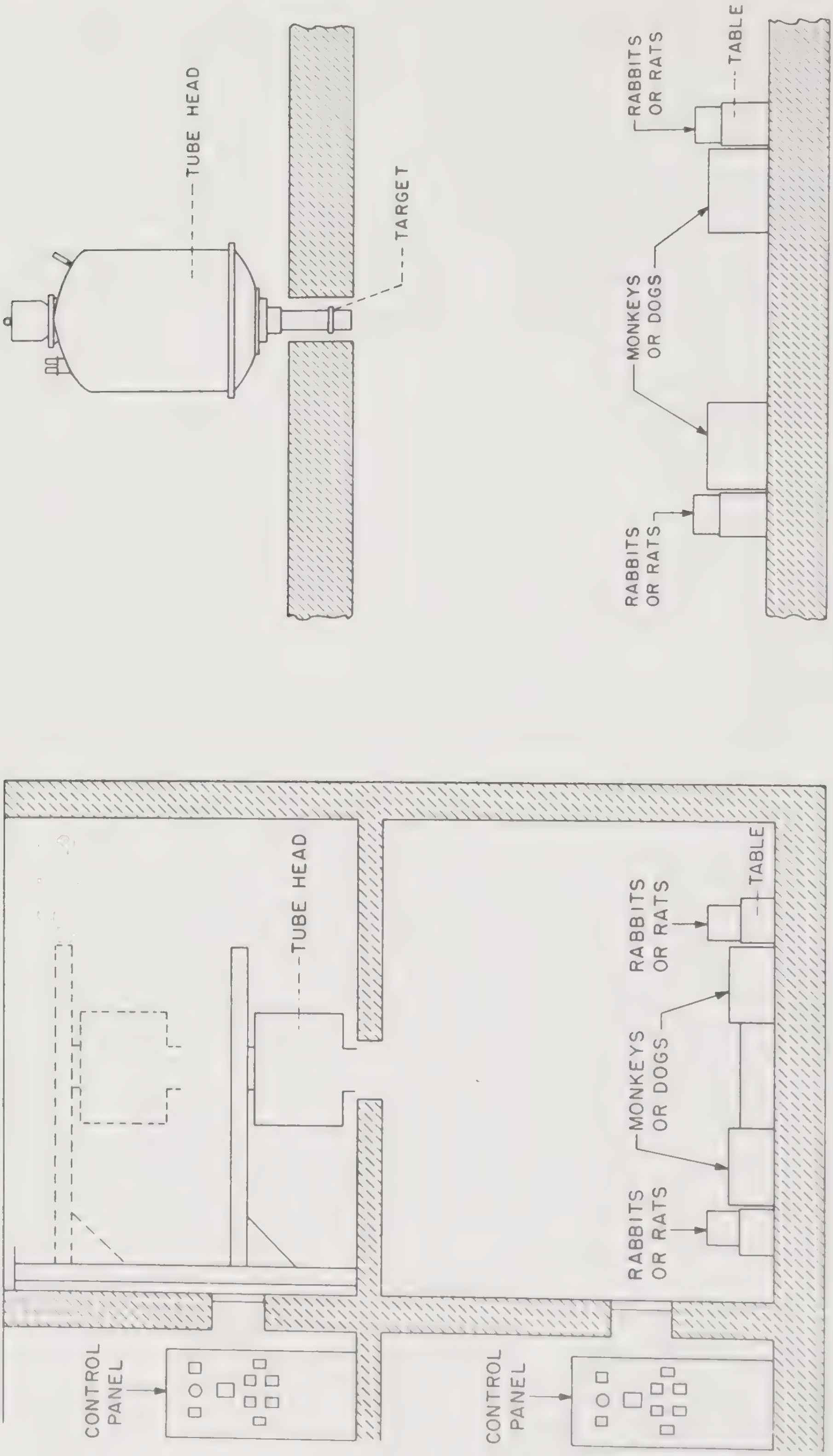

Fig. 9.1 — Arrangement of the animal treatment cages in the X-ray rooms. (a) 250-kv room. (b) 1000-kv room.

the X-ray tubes. Since comparable doses were being given to the various species, the treatment of rats and dogs could proceed simultaneously. This was also possible with monkeys and rabbits. Dogs receiving 3 and 6 r/day constituted an exception since these dosages were not given to other species.

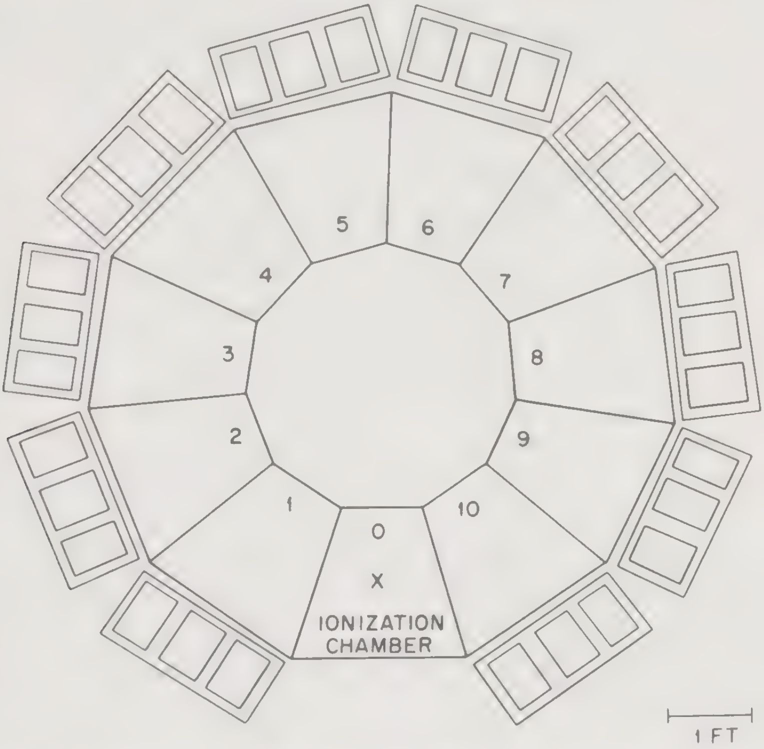

Fig. 9.2—Arrangement of the animal treatment cages, top view.

The dosages referred to throughout the test are those measured in air by Victoreen ionization chambers and electroscopes. Dosages were delivered by timing exposures during the first 6 months and by Victoreen integrons during the remainder of the 2 years. Recalculation of the treatment times and check measurements on the integrons were made routinely once a week or oftener if necessary. In making these measurements, the ionization chamber was placed in the vacant cage space 1 ft above the floor (Fig. 9.2). All dosages therefore refer to dosages measured at this position. The question of how representative the measurements at this point are of the amount that was delivered to the animals is discussed in Sec. 3. The average doses and treatment times are shown in Table 9.4.

The standard errors for these mean values were quite low, and it is clear from Table 9.4 that (1) the doses stipulated were very closely approximated and (2) these doses were given in times varying in different cases from about 8 to 18 min, i.e., the dosage was varied principally by varying the intensity. Time of treatment was kept substantially constant. Short-time exposures were chosen because it was believed that the greatest biological effect would be observed at a given dosage level if this dose were given at relatively high intensity.

Table 9.4—Average Measured Doses and Treatment Times

Dose, r (stipulated)	Average dose, r (measured)	Average time, min	Number of measurements
Rats and Dogs; 1000-kv X Radiation			
0.1	0.0993 ± 0.0004	12.04	92
0.5	0.495	12.58	78
1.0	1.004	10.813	79
3.0	2.98	10.59	104
6.0	5.96 ± 0.030	10.78	107
10.0	9.93 ± 0.033	18.26	111
Monkeys and Rabbits; 1000-kv X Radiation			
0.1	0.1001 ± 0.0006	11.96	64
0.5	0.498	12.46	88
1.0	1.013	10.45	53
10.0	9.96 ± 0.047	18.46	54
Rats and Dogs; 250-kv X Radiation			
0.1	0.102 ± 0.008	8.27	146
0.5	0.499	10.36	148
1.0	1.041	9.98	98
3.0	2.97	9.33	129
6.0	5.96 ± 0.033	10.34	129
10.0	10.03 ± 0.049	18.28	172
Monkeys and Rabbits; 250-kv X Radiation			
0.1	0.1015 ± 0.0007	8.46	100
0.5	0.496	10.33	97
1.0	1.027	9.67	51
10.0	10.15 ± 0.102	8.58	94

3. DISTRIBUTION OF RADIATION AMONG CAGES

Exposure of animals was preceded by exhaustive tests of intensity distributions around the circles in which the cages were arranged.

These tests were repeated on occasion, for instance, after X-ray tubes were replaced. An example of the method used will be given since it is one characterized by great precision and efficiency.

Five Victoreen ionization chambers (A to E) of 250 mr capacity were available. This made possible the simultaneous comparison of five different points in the field of radiation. One of these points (0) was the point used for calibration of the integron. The other four points (2, 4, 7, and 9) were points in the center of dog cages which were distributed in a circular pattern around the field (Fig. 9.2). The problem was to find the true dose at positions 2, 4, 7, and 9 in terms of the stated dose (position 0). This was most efficiently solved by making five trials with the five chambers, or 25 measurements in all. One further restriction placed on the arrangement was that a given chamber was placed only once in a given position. Thus all the combinations of chambers and positions were tried. This repetition of measurement would be unnecessary if all the chambers gave the same reading after a given exposure. As shown below, the largest component of error present in this instance was that due to differences between the readings of chambers. The combinatorial properties stated above are evidently those of a Latin square.

Table 9.5—Arrangement of Ionization Chambers

	Positions				
Trials	0	2	4	7	9
I	C	E	B	A	D
II	D	C	E	B	A
III	E	A	C	D	B
IV	A	B	D	E	C
V	B	D	A	C	E

In practice, one of the 161,280 possible 5-by-5 Latin squares was selected at random[6] and is given in Table 9.5. This square shows the position in the circle for each of the five chambers A to E during each of the five trials. Notice that, in Table 9.5, each letter (chamber) appears once in each row and once in each column. This fact is the basis of the partition of error into its several components.

Five independent trial exposures (250 kv, 15 ma; 20 sec) were given with the chambers assigned as indicated in Table 9.5. The results in milliroentgens are given in Table 9.6.

The analysis of variance appropriate to the Latin square yields the results shown in Table 9.7.

The mean square for error represents the variance due to error per se in making a single measurement. Its small value is a consequence of the elimination of the other "systematic" sources of error. All the other sources of variations are very significant compared to the pure error component, the variance between positions being well beyond the range of ordinary chance (P < 0.1 per cent). Note that the variance between chambers is the largest single component (greatest mean square). This means that the radiation field is more uniform than the chambers used to measure it and that the output of the machine in five successive trials also varied less than the chambers.

Table 9.6—Trial Exposures

	Positions				
Trials	0	2	4	7	9
I	179	174	175	183	174
II	173	175	175	174	178
III	175	175	175	173	171
IV	182	169	176	178	175
V	173	168	178	175	173
Mean	176.4	172.2	175.8	176.6	174.2

Table 9.7—Results of Analysis of Variance

	Sums of squares	Degrees of freedom	Mean square
Trials	44.96	4	11.24
Chambers	149.36	4	37.34
Positions	68.16	4	17.04
Error	14.48	12	1.21
Total	276.96	24	

Since the dosage as measured at position 0 was the "stated" dose to the animals, the dosages at other points ("actual" doses) may be calculated, if required, from the above-mentioned data by subtracting the percentages given in Table 9.8. Positions 4 and 7 are not significantly different from position 1. Positions 2 and 9 require only a small correction, which may be neglected.

Comparable data for the 1000-kv machine were obtained. A sample set of observations is given in Table 9.9. Analysis of these data leads to the conclusion that, with the 1000-kv machine, no significant difference could be found between different cages from the reference point at which the dosage was standardized. The real contribution of this method is apparent from the fact that relative dosage determinations are possible with an accuracy of 6 parts in 1000 employing instruments whose responses differ from one another by about 4 per cent.

Table 9.8—Percentages of Stated Dose To Be Subtracted To Obtain True Dose at Each Position

Position	Percentage
2	2.4
4	None
7	None
9	1.2

Table 9.9—Trial Exposures with 1000-kv Machine

	Position				
Trial	0	2	4	7	9
I	104	98	102	99	100
II	131	129	129	132	135
III	131	133	128	127	128
IV	125	125	130	125	128
V	125	128	125	130	125
Mean	123.2	122.6	122.8	122.6	123.2

Successive tests inside rat cages were also carried out to ensure that errors of intensity between positions were always less than ±3 per cent of the stated dose.

In addition to the techniques outlined above, routine use was made of dental film and lead step wedges (a suggestion of G. Failla and W. F. Bale) which were included with appropriate lead data and code numbers with each group of animals treated. These were developed weekly and tended to give an over-all check on the order of magnitude of dosage. This was a precaution against interchanging animal groups in treatment. In fact, five such cases were discovered in this way, all of which were negligible except one in which half the 0.1-r monkeys received 8 r in excess of their proper dose. The chief benefit derived from the practice, however, was to make animal handlers and X-ray technicians very careful in carrying out their work.

4. COMPARISON OF 1000- AND 250-KV RADIATION DETERMINED BY PHANTOM MEASUREMENTS

Because of the need for more than a mere estimate of the spatial distribution of the X-ray energy in the treated animals, especially the dog, a Presdwood phantom was constructed, and ionization measurements were made at various depths and locations within the phantom. In addition, half-value measurements were made after the beam had penetrated increasing thicknesses of Presdwood. The results of this investigation are shown in Fig. 9.3.

The Presdwood phantom had to be drilled at various points for introduction of the ionization chamber, and naturally only a limited number of holes could be made. It was decided to attempt to supplement the data obtained by ionization instruments by inserting an X-ray film vertically and longitudinally between the laminations of the Presdwood dog with another one vertical but at right angles to the first. Measurements of the density of the film were made with an Ansco-Sweet direct-reading densitometer. The densitometer measurements were of the same order of magnitude as the ionization measurements, but because of the inherent difficulties in using a film density as a measure of X-ray intensity, the ionization measurements only are included in this chapter.

Half-value Layer (HVL) Measurements. The 250-kv machine is operated at a target-skin distance (TSD) of 10 ft 10 in. In order to obtain a uniform intensity of radiation at the level at which the animals are treated, a parabolic Al filter was cast which measured 1 in. in thickness at the center and ⅛ in. at the edge with a diameter of 9 in. No additional filtration was used except when it was desired to reduce the intensity to a value sufficient to supply radiation at a rate of about 0.01 r/min for one group of animals. In this case a ½-mm filter of copper was added to the parabolic filter. The beam as applied for treatments gave a half-value measurement of 2.10 mm Cu. In order to determine whether the beam became increasingly hard as it passed through increasing thicknesses of the dog, a series of HVL measurements was made after the beam had traversed 1-in. increments of the Presdwood phantom.

Unusual values of the HVL measurements gave rise to the suspicion that scattered radiation from the floor and walls of the room was contributing considerable radiation to the chamber during the long exposures necessary. Accordingly, a complete lead shield was con-

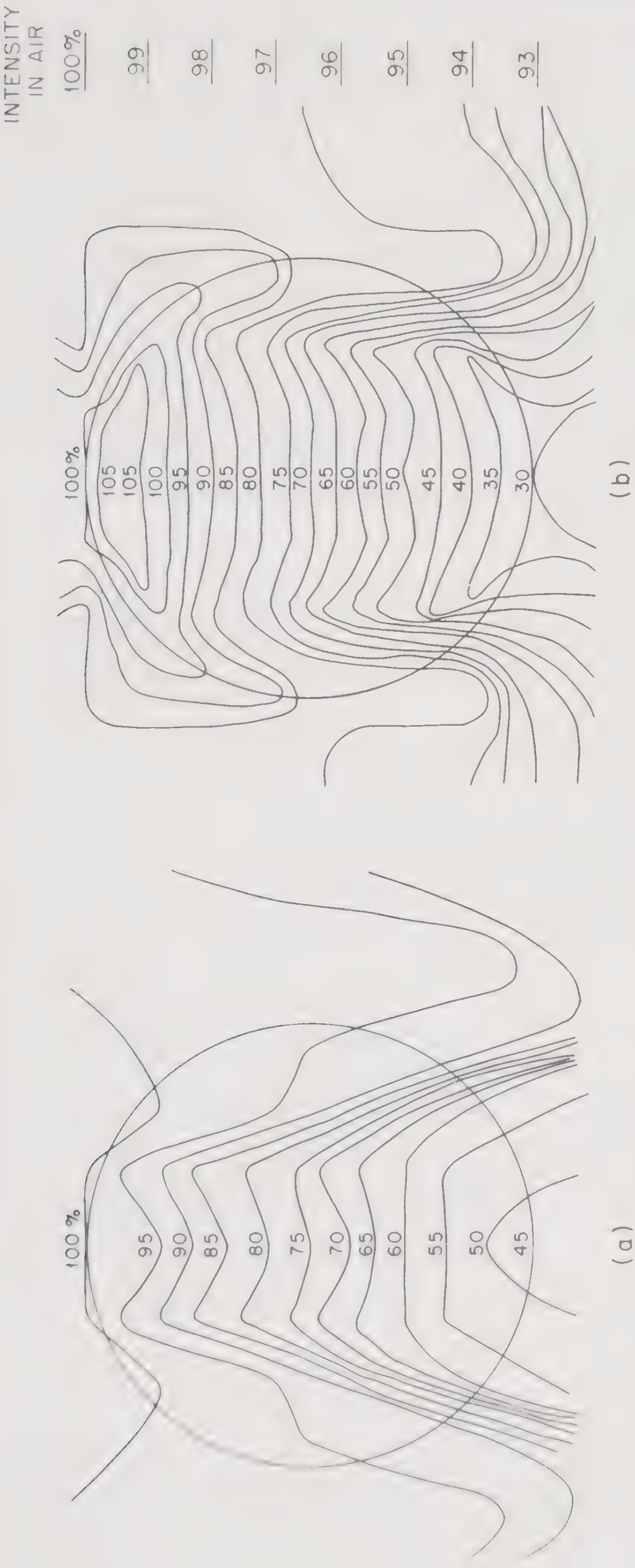

Fig. 9.3—Isodose curves for X radiation in a phantom dog. (a) 1000-kv radiation. (b) 250-kv radiation.

structed, and the HVL measurements were repeated. It was noted that shielding the measuring chamber from scattered radiation from the floor and walls reduced the intensity of the treatment beam by 11.6 per cent. However, the depth-dose curves must of necessity include such scattered radiation since the animals were treated in the open room under such conditions that the scattered radiation could not be excluded. Shielding the ionization chamber from the direct beam gave a reading of scattered radiation alone in agreement with the amount implied by the previous method of measurement. HVL measurements of the scattered radiation were made, giving 0.93 mm Cu, compared with 2.10 mm Cu for the primary beam. The HVL was found to be practically unchanged after the beam had passed through 5 in. of the Presdwood phantom.

The 1000-kv beam had a HVL equal to 5.12 mm Pb and 10.7 mm Cu. The depth-dose curves on this beam reveal several points of interest (Fig. 9.3).

1. The backscatter is so small that, except near the edge along the upper lateral surface, nowhere within the phantom is the intensity higher than at the surface. This is in marked contrast with the 250-kv depth-dose curves.

2. The depth dose is generally higher than in the case of the 250-kv radiation. The emergent intensity is 45 per cent of the surface intensity, whereas, in the 250-kv curves, it is only about 30 per cent of the surface intensity.

The X-ray field strength of the 250-kv radiation is shown in Fig. 9.3b as it appears at the level of, but without the presence of, an animal. The intensity decreases with distance, within the limits of error of the instruments, according to the inverse-square law. The introduction of a dog distorts this field in a manner similar to the placing of a piece of iron in a uniform magnetic field. In actual practice the dogs had a limited freedom of movement, i.e., they were able to stand or lie down. It is expected that the depth-dose measurements as made in the phantom only approximate the conditions to be found in the living animal because of wide variations of shape and differences in density of the various organs.

The results obtained on analysis of this experiment are presented in the following four chapters.

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Chapter 10*

EFFECTS OF CHRONIC EXPOSURE TO X RADIATION ON GROWTH AND SURVIVAL

By Robert D. Boche

1. SURVIVAL

Because radiation must affect the life tables of exposed populations if its effects are of major importance to the individuals making up the population, the data on survival have been given careful study and analysis. The results can be stated in a variety of ways: for example, death rates per week, proportions dying per week; median survival time, time required for half the animals to die; or mean survival time, average life expectancy. Extensive theoretical treatment can be made to yield various mathematical functions for description of population behavior under the influence of chronic irradiation, but the constants of such equations are usually not suitably related to the biological effects in the individual, i.e., such treatment usually provides, at most, a formal description of events. For this reason an elaborate analysis is not given here.

Tables 10.1 to 10.4 give the vital statistics for each group of animals and show complete data for each animal dying during the period of radiation. These tables give the sex, number of treatments, and calendar weeks of survival for each animal.

Survival in rats is represented graphically in Fig. 10.1, which is based on the data of Table 10.1. For some purposes the data are most clearly represented by the transformation of cumulative percentages to probits according to the method of Bliss.[1] Figure 10.2 shows the rat data plotted as probits. The median death times are read as the times at which the various curves cross the probit 5

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* Based on Report MDDC-204 (II-188-5936).

Table 10.1 — Survival Data for Rats Exposed to Chronic X Radiation

Controls
Total Treatments, 24,462
Survivors, 4 Males, 10 Females

Animal No.	Sex	Number of treatments	Survival time, weeks
20	M	118	20.0
21	M	214	36.0
23	M	230	38.5
29	M	238	40.0
27	M	240	40.5
15	M	256	43.0
45	F	256	43.0
7	F	298	50.0
28	M	302	50.5
22	M	351	59.0
30	M	369	62.0
1	M	387	65.0
3	M	390	65.5
35	F	408	68.5
19	M	423	71.0
18	M	451	75.5
42	F	455	76.5
17	M	459	77.0
13	M	463	77.5
43	F	481	80.5
4	M	503	84.5
6	F	509	85.5
46	F	523	87.5
49	F	523	87.5
32	F	531	89.0
36	F	543	91.0
50	F	565	95.0
26	M	567	95.0
37	F	605	101.5
11	M	610	102.5
12	F	610	102.5
16	M	610	102.5
31	F	613	103.0
41	F	614	103.0
47	F	619	104.0
2	M	620	104.0

0.1 r/Day, 1000-kv Radiation
Total Treatments, 24,994
Survivors, 10 Males, 8 Females

Animal No.	Sex	Number of treatments	Survival time, weeks
11	M	158	26.5
4	M	225	38.0
22	F	252	42.5
10	F	266	44.5
30	M	270	45.5
3	M	291	49.0
26	M	294	49.5
7	F	334	56.0
1	M	338	57.0
46	F	346	58.0
27	M	347	58.5
16	M	353	59.5
21	F	403	67.5
19	F	410	69.0
31	F	417	70.5
2	M	442	74.0
8	F	465	78.0
17	M	470	79.0
15	M	488	82.0
20	F	490	82.0
28	M	510	85.5
48	F	513	86.0
32	F	514	86.0
47	F	526	88.0
38	M	552	92.5
37	M	560	94.0
34	F	575	96.5
41	F	577	97.0
25	M	585	98.0
33	F	594	99.5
5	M	613	103.0
24	M	620	104.0

0.1 r/Day, 250-kv Radiation
Total Treatments, 23,693
Survivors, 4 Males, 5 Females

Animal No.	Sex	Number of treatments	Survival time, weeks
19	M	66	11.5
30	M	205	34.5
8	F	229	38.5
33	F	248	41.5
43	F	248	41.5
29	M	292	49.0
49	F	304	51.0
44	F	328	55.0
10	F	341	57.5
22	M	360	60.5
36	F	369	62.0
37	F	375	63.0
45	F	377	63.5
11	M	398	67.0
48	F	419	70.5
4	M	420	70.5
7	F	424	71.0
28	M	429	72.0
46	F	429	72.0

Table 10.1 — (Continued)

0.1 r/Day, 250-kv (Continued)

Animal No.	Sex	Number of treatments	Survival time, weeks
16	M	444	74.5
35	F	452	76.0
40	F	463	77.5
39	F	472	79.0
24	M	508	85.0
18	M	509	85.5
23	M	522	87.5
1	M	523	87.5
25	M	527	88.5
26	M	545	91.5
2	M	549	92.0
13	M	549	92.0
27	M	552	92.5
17	M	562	94.5
14	M	563	94.5
15	M	566	95.0
50	F	572	96.0
12	M	576	96.5
32	F	576	96.5
31	F	591	99.0
9	F	602	101.0
47	F	611	102.5

0.5 r/Day, 1000-kv Radiation
Total Treatments, 21,854
Survivors, 4 Males, 7 Females

Animal No.	Sex	Number of treatments	Survival time, weeks
40	F	145	24.5
5	M	168	28.5
45	F	169	28.5
24	M	212	35.5
19	M	220	37.0
38	F	229	38.5
3	M	283	47.5
39	F	285	48.0
9	F	301	50.5
30	M	301	50.5
15	M	314	52.5
1	M	314	52.5
2	M	320	54.0
33	F	323	54.0
23	M	329	55.5
10	F	329	55.5
6	F	355	59.5
22	M	363	61.0
31	F	369	62.0
37	F	379	63.5
11	M	385	64.5
34	F	417	70.0
32	F	425	71.5
43	F	438	73.5
42	F	443	74.5
35	F	459	77.0
14	M	462	77.5
49	F	471	79.0
18	M	481	80.5
25	M	482	81.0
12	M	494	83.0
21	M	495	83.0
29	M	499	83.5
27	M	519	87.0
17	M	547	92.0
13	M	556	93.5
20	M	561	94.0
44	F	562	94.5
50	F	608	102.0

0.5 r/Day, 250-kv Radiation
Total Treatments, 24,314
Survivors, 6 Males, 8 Females

Animal No.	Sex	Number of treatments	Survival time, weeks
4	M	212	35.5
1	M	219	37.0
23	M	225	38.0
9	F	256	43.0
27	M	268	45.0
30	M	277	46.5
2	M	279	47.0
41	F	299	50.0
35	F	311	52.0
31	F	319	53.5
16	M	354	59.5
5	M	388	65.0
44	F	393	66.0
29	M	399	67.0
28	M	435	73.0
12	M	437	73.5
15	M	443	74.5
17	M	447	75.0
32	F	448	75.0
49	F	449	75.5
18	M	477	80.0
36	F	485	81.5
26	M	491	82.5
50	F	503	84.5
45	F	511	85.5
20	M	521	87.5
11	M	535	90.0
46	F	554	93.0

Table 10.1 — (Continued)

Animal No.	Sex	Number of treatments	Survival time, weeks
0.5 r/Day, 250-kv (Continued)			
21	M	561	94.0
33	F	572	96.0
14	M	574	96.5
47	F	581	97.5
6	F	584	98.0
37	F	588	98.5
43	F	592	99.5
34	F	619	104.0
1.0 r/Day, 1000-kv Radiation Total Treatments, 23,459 Survivors, 6 Males, 2 Females			
9	F	192	32.5
10	F	256	43.0
3	M	269	45.0
16	M	281	47.0
21	M	274	46.0
5	M	287	48.0
6	F	306	51.5
2	M	317	53.0
37	F	325	54.5
38	F	343	57.5
48	F	351	59.0
33	F	381	64.0
31	F	388	65.0
44	F	410	69.0
32	F	411	69.0
4	M	417	70.0
42	F	418	70.0
24	M	420	70.5
14	M	426	71.5
11	M	442	74.0
12	M	446	75.0
39	F	459	77.0
43	F	459	77.0
50	F	464	78.0
46	F	469	78.5
30	M	470	79.0
23	M	471	79.0
15	M	475	79.5
7	F	489	82.0
47	F	501	84.0
8	F	501	84.0
36	F	513	86.0
40	F	536	90.0
49	F	549	92.0
45	F	576	96.5
25	M	577	97.0
29	M	580	97.5
1	M	594	99.5
26	M	600	100.5
28	M	601	101.0
34	F	618	104.0
20	M	621	104.5
1.0 r/Day, 250-kv Radiation Total Treatments, 23,494 Survivors, 6 Males, 6 Females			
16	M	44	7.5
44	F	216	36.5
9	F	283	47.5
43	F	284	47.5
5	M	294	49.5
27	M	304	51.0
12	M	323	54.0
4	M	330	55.5
33	F	354	59.5
50	F	363	61.0
7	F	369	62.0
6	F	370	62.0
3	M	374	63.0
23	M	381	64.0
34	F	393	66.0
41	F	398	67.0
18	M	405	68.0
22	M	411	69.0
1	M	417	70.0
24	M	420	70.5
15	M	422	71.0
21	M	429	72.0
47	F	432	72.5
39	F	442	74.0
20	M	443	75.0
42	F	471	79.0
40	F	484	81.0
32	F	497	83.5
36	F	498	83.5
29	M	514	86.0
26	M	544	91.5
28	M	557	93.5
48	F	578	97.0
8	F	581	97.5
31	F	597	100.0
37	F	599	100.5
11	M	601	101.0
13	M	608	102.0

Table 10.1 — (Continued)

Animal No.	Sex	Number of treatments	Survival time, weeks	Animal No.	Sex	Number of treatments	Survival time, weeks
10.0 r/Day, 1000-kv Radiation Total Treatments, 9287 No Survivors				10.0 r/Day, 250-kv Radiation Total Treatments, 9256 No Survivors			
18	F	191	32.0	10	M	32	5.5
10	M	277	46.5	4	M	202	34.0
14	M	281	47.0	19	F	262	44.0
2	M	298	50.0	14	M	287	48.0
6	M	314	52.5	5	M	307	51.5
3	F	323	54.0	13	M	328	55.0
16	F	328	55.0	3	F	333	56.0
24	F	334	56.0	6	M	344	58.0
7	M	334	56.0	23	F	373	62.5
15	M	341	57.5	25	F	375	63.0
1	M	343	57.5	8	M	376	63.0
4	F	357	60.0	21	F	383	64.5
12	M	369	62.0	9	M	386	65.0
22	F	375	63.0	12	M	387	65.0
25	F	381	64.0	15	M	387	65.0
20	F	395	66.5	22	F	387	65.0
5	F	395	66.5	2	F	407	68.5
17	F	405	68.0	1	F	430	72.0
8	M	405	68.0	11	M	443	74.5
11	M	428	72.0	16	F	447	75.0
23	F	430	72.0	17	F	449	75.5
21	F	462	77.5	24	F	453	76.0
13	M	465	78.0	18	F	460	77.0
9	M	525	88.0	20	F	477	80.0
19	F	531	89.0	7	M	541	91.0

(50 per cent mortality). The median death times determined in this manner are shown in Table 10.5.

Two other features of the graph in Fig. 10.2 may be noted: (1) the slopes of the probit treatment curves increase slightly with each increase in daily dose, and (2) the 10 r/day curve is discontinuous at 300 treatments or approximately 1 year of radiation. The frequency of death among the 10 r/day animals was the same as the controls throughout the first year of radiation, after which time a marked increase in death rate was noted. This first year may be regarded as a lag phase or a period of induction. Although it would be difficult to insist on statistically significant differences between controls on the one hand and 0.1 and 0.5 r on the other, they will be treated here as if the difference were real because the chance of obtaining four slopes which increase in the order observed is only 1 in 24. There is, of

Table 10.2 — Survival Data for Dogs Receiving Chronic X Radiation

Radiation		Animal No.	Sex	Number of treatments	Survival time, weeks	Number of survivors		Total treatments
r/day	kv					Males	Females	
Controls		10	F	551	91.8	4	4	5527
0.1	1000*					3	7	6220
0.1	250	1	F	603	101	4	5	6201
0.5	1000*					3	7	6220
0.5	250*					4	6	6220
1.0	1000	3	M	533	90.5	2	6	6060
		10	M	551	92.0			
1.0	250	9	M	513	86.0	6	3	6111
10.0	1000	1	F	120	19.0	2	1	2985
		2	F	311	51.0			
		3	F	134	22.5			
		4	F	254	43.0			
		5	M	359	60.0			
		6	M	124	21.5			
		8	M	183	31.5			
10.0	250	1	M	130	21.5	0	1	1818
		2	F	132	22.0			
		3	F	110	18.5			
		4	F	127	21.0			
		5	F	144	24.0			
		7	M	107	19.0			
		8	M	266	45.5			
		9	M	115	20.5			
		10	M	187	32.5			
3.0	1000	1	M	393	66.0	2	2	2393
3.0	250	1†	M	435	73.5	0	3	1921
		2	M	421	71.0			
6.0	1000	1	F	491	83.0	0	1	2088
		2	M	384	65.0			
		3	M	344	58.0			
		4	M	369	62.0			
6.0	250	4	F	218	37.0	3	1	2218

* No deaths in these groups.

† Accidental death, not included in calculations.

Table 10.3—Survival Data for Rabbits Exposed to Chronic X Radiation

Radiation		Animal No.	Sex	Number of treatments	Survival time, weeks	Number of survivors		Total treatments
r/day	kv					Males	Females	
Controls		11	M	54	9.0	7	3	4203
		1	M	59	10.0			
		15	F	163	27.5			
		14	F	255	43.0			
		5	M	265	44.5			
		2	M	307	51.5			
0.1	1000	3	M	13	2.0	2	4	2410
		2	M	18	3.0			
		5	M	259	43.5			
		10	F	260	43.5			
0.1	250	3	M	64	12.0	2	2	2173
		2	M	108	19.5			
		7	F	123	22.0			
		9	F	131	23.0			
		1	M	242	42.0			
		6	F	265	45.5			
0.5	1000	4	M	16	3.5	3	4	2537
		10	F	57	10.5			
		5	M	294	50.0			
0.5	250	6	F	47	9.0	4	2	2258
		1	M	87	15.5			
		8	F	93	16.5			
		10	F	171	29.5			
1.0	1000	6	F	32	5.5	4	1	1947
		7	F	41	7.0			
		8	F	42	7.0			
		3	M	60	10.0			
		10	F	222	37.5			
1.0	250	10	F	159	26.0	5	3	2802
		9	F	163	27.5			
10.0	1000	1	M	32	6.0	1	3	2394
		7	F	173	29.5			
		3	M	177	30.0			
		4	M	226	38.5			
		9	F	255	44.0			
		2	M	291	49.0			
10.0	250	6	F	20	4.0	3	1	1953
		9	F	43	8.0			
		5	M	57	10.0			
		10	F	139	24.0			
		8	F	144	24.5			
		1	M	310	52.5			

Table 10.4 — Survival Data for Monkeys Exposed to Chronic X Radiation

Radiation		Animal No.	Sex	Number of treatments	Survival time, weeks	Number of survivors		Total treatments
r/day	kv					Males	Females	
Controls		4^2	F	138	23	2	1	2170
		5^2	M	166	27.5			
0.1	1000	4		18	4.0	1	0	3844
		10		25	5.0			
		6	F	39	7.5			
		4^2	M	64	10.5			
		5	F	133	23.0			
		1	M	236	40.5			
		9	F	295	50.0			
		3	F	317	54.0			
		2	F	352	60.0			
		7	F	546	92.5			
		8	M	602	102.0			
		10^2	M	595	100.0			
0.1	250	10		6	1.0	1	2	4077
		9		7	1.5			
		6		9	2.0			
		2		9	2.0			
		8		20	4.5			
		4	F	107	19.0			
		8^2	F	168	28.0			
		2^2	F	264	44.0			
		1	F	329	56.0			
		5	M	329	56.0			
		6^2	M	445	71.5			
		10^2	F	518	86.5			
0.5	1000	8		7	1.5	0	1	2923
		2		9	2.0			
		4		9	2.0			
		6		11	3.0			
		1		20	4.5			
		5	M	68	12.5			
		2^2	F	20	3.5			
		7	F	96	17.0			
		10	M	106	18.5			
		1^2	M	178	29.5			
		8^2	M	200	33.5			
		4^2	M	220	36.5			
		3	M	367	62.5			
		6^2	M	462	76.5			
		5^2	F	528	89.0			
0.5	250	4		5	1.0	2	0	3925
		2		7	1.5			
		8		9	2.0			
		9		9	2.0			
		5		9	2.0			
		7		9	2.0			
		4^2		21	4.5			
		1	F	171	29.5			
		8^2	F	114	19.0			
		7^2	M	165	27.5			
		3	M	227	39.0			
		2^2	F	310	52.0			
		5^2	M	429	71.5			
		10	M	586	99.0			
		4^3	M	610	102.5			

Table 10.4 — (Continued)

Radiation		Animal No.	Sex	Number of treatments	Survival time, weeks	Number of survivors		Total treatments
r/day	kv					Males	Females	
1.0	1000	3		9	2.0	1	1	3684
		9		9	2.0			
		4		10	2.5			
		1		11	3.0			
		5		14	3.5			
		10		18	4.0			
		8		23	5.0			
		7	M	51	9.5			
		5[2]	F	63	12.5			
		4[2]	M	75	12.5			
		9[2]	F	74	13.5			
		6	F	297	52.5			
		8[2]	F	252	42.0			
		10[2]	M	478	80.0			
		7[2]	M	502	84.0			
		3[2]	M	554	93.0			
1.0	250	5		4	0.5	0	2	3362
		2		5	1.0			
		3		8	1.5			
		7		9	2.0			
		10		9	2.0			
		4		9	2.0			
		5[2]		21	4.5			
		1	M	44	8.5			
		3[2]	M	18	3.0			
		9	M	79	14.0			
		8	M	156	27.0			
		6	M	219	37.5			
		7[2]	M	169	28.0			
		4[2]	F	267	44.5			
		10[2]	F	545	91.5			
		5[3]	F	556	93.0			
10.0	1000	1		14	3.5	0	0	917
		5		15	3.5			
		2		24	5.0			
		4	F	84	15.0			
		1[2]	M	51	8.5			
		2[2]	F	128	21.5			
		3	F	200	34.5			
		5[2]	M	401	67.0			
10.0	250	3		9	2.0	0	0	692
		2		14	3.5			
		1	M	78	14.0			
		5	M	81	14.5			
		2[2]	M	43	8.0			
		4	M	108	19.0			
		3[2]	M	359	60.0			

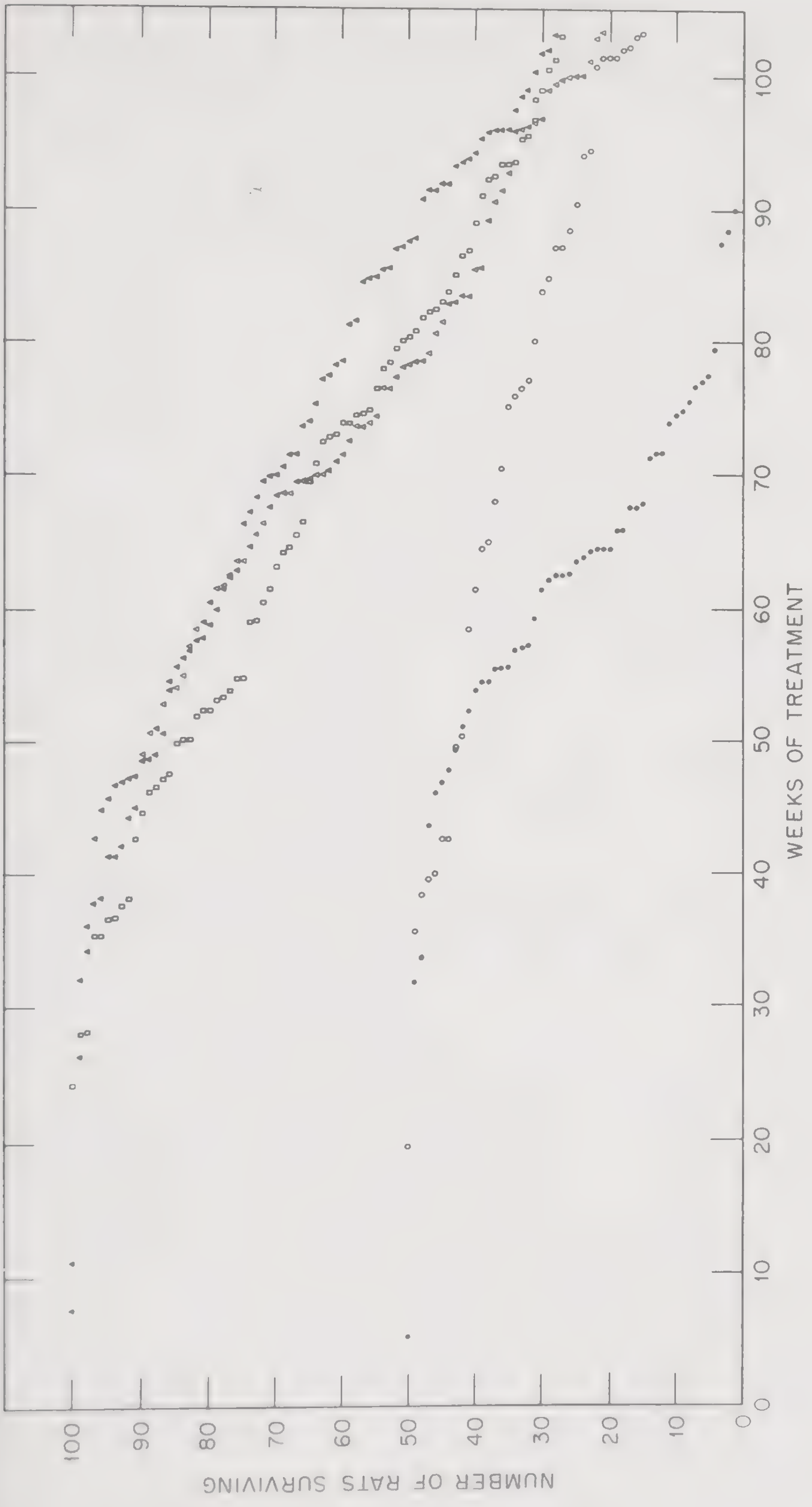

Fig. 10.1—Survival of rats chronically exposed to X radiation. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

course, no question of effects at 10 r/day. From a study of the behavior of males and females and of 1000-kv radiation as compared to 250-kv radiation, no evidence of a significant difference due to sex or voltage could be discovered. An effect of sex has not been observed in studies reported in Chap. 1 with acute radiation, but it is well known that, in acute exposures, higher voltages are somewhat less effective biologically than lower ones. In the subsequent analyses the effect of differences in voltage will, in general, be ignored because the experiments do not involve sufficient numbers of animals to clarify the point and there is general agreement between results of the two series, 250 and 1000 kv.

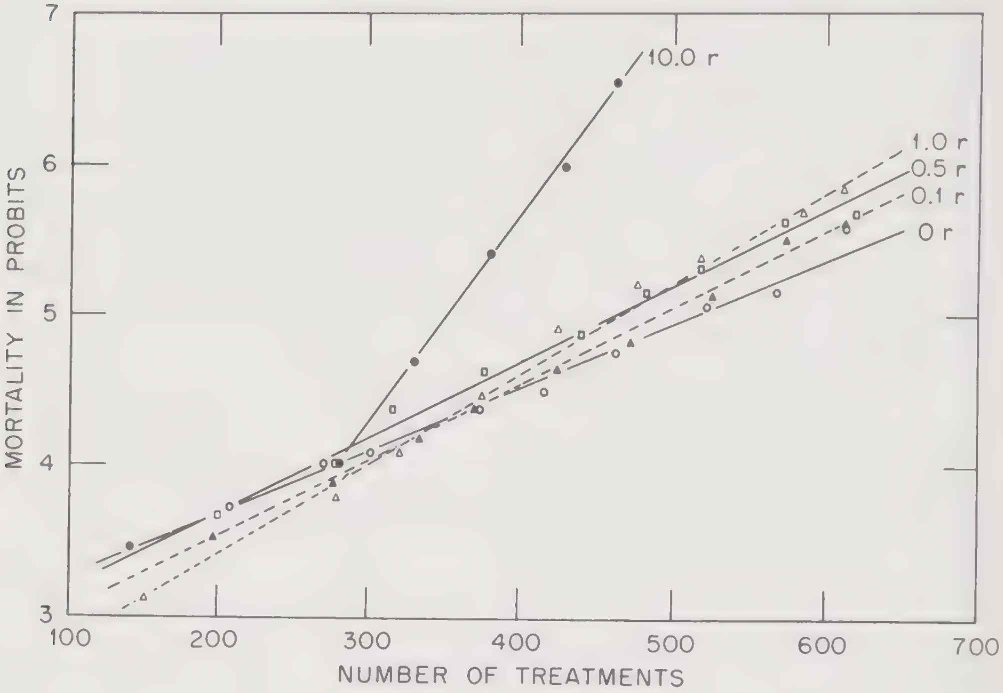

Fig. 10.2—Mortality in probits of rats exposed to various levels of chronic X radiation in relation to number of treatments. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

Table 10.6 illustrates the point that, with long-lived species, no effects on survival are shown at doses below 1 r/day when the experiments are conducted for only a fraction of the animal's normal life. This is illustrated also for rabbits and monkeys in Tables 10.7 and 10.8.

Table 10.5—Median Number of Treatments and Time of Survival for Rats

Dosage group, r	Number of treatments for 50% deaths	Median death time, weeks
0	515	85.8
0.1	490	81.7
0.5	460	76.7
1.0	462	76.9
10.0	350	58.3

Table 10.6—Mortalities in Dogs Chronically Exposed to Radiation

Level, r	Number of animals	Number of deaths	Mortality, %	Duration of exposure, months
0	9	1	11	
0.1	20	1	5	24
0.5	20	0		24
1.0	20	3	15	24
3.0	9*	2	22	19.2
6.0	10	5	50	19.2
10.0	20	16	80	19.2

*One dog which died an accidental death is not included here.

Table 10.7—Mortalities in Rabbits Chronically Exposed to 1 Year of X Radiation

Level, r	Number of animals	Number of deaths	Mortality, %
0	16	6	38
0.1	20	10	50
0.5	20	7	35
1.0	20	7	35
10.0	20	12	60

It is obvious from Table 10.7 that no effect of radiation on survival in the rabbit is discernible at levels of 1 r/day or less. This is also true of monkeys, as is shown in Table 10.8.

Survival data on the monkeys are almost valueless because of the very high incidence of tuberculosis in the colony, which was responsible for the death of most of the monkeys. Many lived only a few weeks after the start of the experiment. An attempt to correct for these brief survivals has been made in column 5 of Table 10.8, which represents the result of disregarding all animals that lived for less than 100 treatments. As can be seen, the results are not improved.

Table 10.8—Mortalities in Monkeys Exposed to Chronic X Radiation for 2 Years

Level, r	Number of animals	Number of deaths	Mortality, %	Correction, %
0	5	2	40	40
0.1	28	24	86	79
0.5	33	30	91	83
1.0	36	32	89	73
10.0	15	15	100	100

2. CONCEPT OF MORTALITY PER ROENTGEN

From Tables 10.1 to 10.4, further information may be obtained about the nature of the effect of radiation on survival in cases where the experiment is carried out over an appreciable fraction of the natural life of the animal or the radiation levels are high enough for significant numbers of animals to die as compared to controls. Since the experiment for rats was carried on for 622 treatments, the numbers of treatments for all dying animals may be summed, and the product of 622 times the number of survivors in each group may be added to this sum. This represents for each group the total number of rat treatments given. If the total number of deaths in a group is divided by the total number of rat treatments given, the quotient represents the average proportion of animals dying per treatment for the whole experiment. This average proportion is one in which the individual estimates for each treatment interval have been weighted in proportion to the number of animals alive during that interval. For experiments in which all animals die, the proportion dying per treatment, which is called the "death rate per treatment," is merely the reciprocal of the average survival time. For example, if the average survival time for a group were 400 treatments, the average death rate per treatment would be 0.0025. The average death rates per treatment for rats are given in Table 10.9. These relations are shown in Fig. 10.3, from which it appears that there is an approximately linear increase in the average death rate per treatment with the amount of radiation given per treatment. This suggests that the increase in death rate per treatment as compared to the natural rate divided by the dosage per treatment is approximately constant. It is proposed to call this increase the "increase in mortality per roentgen," denoted by α, and its constancy may be seen from Table 10.10, which is derived from Table 10.9. It is surprising not that the increase in the proportion dying per roentgen is not exactly constant but that the de-

Table 10.9 — Average Death Rates per Treatment for Rats

Level, r	Total number of deaths	Total number of treatments	Death rate per treatment
0	36	24,462	1.47×10^{-3}
0.1	73	48,687	1.50×10^{-3}
0.5	75	46,168	1.62×10^{-3}
1.0	80	46,953	1.70×10^{-3}
10.0	50	18,543	2.70×10^{-3}

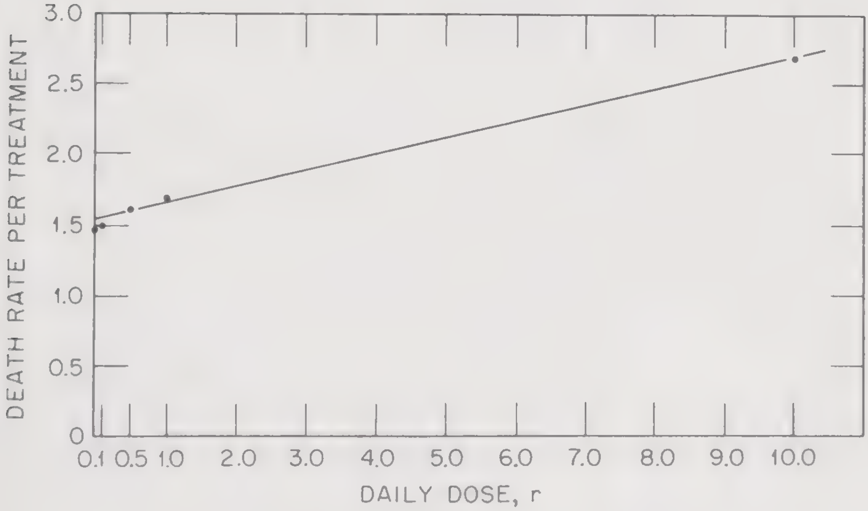

Fig. 10.3 — Relation between the average death rate per treatment and the magnitude of the dose per treatment for rats exposed to chronic X radiation.

viations are no greater than they are, considering that the daily doses were varied over-all by a factor of 100.

It is instructive to examine the survival data on dogs to see whether this relation obtains with this species also. This is done in Table 10.11. Since no effects can be seen at 0.1 or 0.5 r, these groups are pooled with the controls to improve the estimate of the natural mortality per treatment. As before, the α is found by first subtracting the natural rate (here estimated by combining controls and 0.1- and 0.5-r groups) and then dividing by the amount of radiation per treatment.

Table 10.10 — Constancy of α for Rats

Level, r	Difference between control and treated	α
0.1	0.3×10^{-4}	3×10^{-4}
0.5	1.5×10^{-4}	3×10^{-4}
1.0	2.3×10^{-4}	2.3×10^{-4}
10.0	12.3×10^{-4}	1.2×10^{-4}

Table 10.11 — Mortalities per Treatment for Dogs

Level, r	Number of deaths	Number of treatments	Death rate per treatment
0	1	5,283*	
0.1	1	12,421	
0.5	0	12,440	
Total	2	30,144	0.66×10^{-4}
1.0	3	12,171	2.46×10^{-4}
3.0	2	4,314	4.66×10^{-4}
6.0	5	4,306	11.61×10^{-4}
10.0	16	4,803	33.33×10^{-4}

*For a discussion of treatments in control dogs, see Chap. 9.

In Table 10.12 the 10-r group of dogs shows a slightly higher value of α than the others, whereas α for the 10-r group of rats was slightly lower; both fluctuations may be regarded therefore as sampling variations. Since α may be expected to vary with the species and the kind of radiation, it is useful for comparing species and kinds of radiation. In Tables 10.13 and 10.14, α is calculated for rabbits and monkeys,

Table 10.12 — Determination of α for Dogs

Level, r	Excess death rate per treatment over controls and 0.1- and 0.5-r groups	α
1.0	1.8×10^{-4}	1.8×10^{-4}
3.0	4.0×10^{-4}	1.3×10^{-4}
6.0	10.95×10^{-4}	1.8×10^{-4}
10.0	32.6×10^{-4}	3.3×10^{-4}

Table 10.13 — Death Rates per Treatment and Determination of α for Rabbits

Level, r	Number of deaths	Number of treatments	Death rate per treatment	Difference
0	6	4,203	14.3×10^{-4}	
0.1	10	4,583	21.8×10^{-4}	
0.5	7	4,795	14.6×10^{-4}	
1.0	7	4,749	14.7×10^{-4}	
Total	30	18,330	16.4×10^{-4}	
10.0	12	4,347	27.6×10^{-4}	11.2×10^{-4}

$$\alpha = \frac{1}{10}(27.6 - 16.4) \times 10^{-4} = 1.1 \times 10^{-4}$$

Table 10.14 — Death Rates per Treatment and Determination of α for Monkeys

Level, r	Number of deaths	Number of treatments	Death rate per treatment	Difference
0	2	2,170		
0.1	24	7,921		
0.5	30	6,848		
1.0	32	7,046		
Total	88	23,985	36.7×10^{-4}	
10.0	15	1,609	93.2×10^{-4}	56.5×10^{-4}

$$\alpha = \frac{1}{10}(93.2 - 36.7) \times 10^{-4} = 5.7 \times 10^{-4}$$

respectively, from the increase in the death rate per treatment at 10 r/day as compared to all other rates.

Since it has been shown that the concept of α gives an approximate description of survival effects in the rat and dog, it is of interest to see the extent to which it applies in the published data.

Henshaw[2] carried out two small-scale experiments with C_3H mice comparable in many respects to the above experiments. He gave the mice 200-kv **X** radiation 5 days per week in doses of 0, 5, 10, 15, 20, and 25 r/day. The average numbers of weeks of survival and their reciprocals, the death rates per week, are calculated as usual from his data. The difference between the control and experimental death rates per week is found, and the increase in mortality per week is divided by the dose per week to give the α as in the above. Since the time dimension cancels, it is immaterial whether the death rates are expressed per treatment, per day, or per week, provided that the dose is expressed in the same units of time.

Table 10.15—Mortality Statistics Calculated for Mice (Henshaw's Data)

Level, r/week	Average survival, weeks	Death rate per week	Excess over controls	α
0	45.8	21.8×10^{-3}		
25	36.8	27.2×10^{-3}	5.4×10^{-3}	2.2×10^{-4}
50	34.6	28.9×10^{-3}	7.1×10^{-3}	1.4×10^{-4}
75	27.1	36.9×10^{-3}	15.1×10^{-3}	2.0×10^{-4}
100	23.9	41.8×10^{-3}	20.0×10^{-3}	2.0×10^{-4}
125	19.2	52.1×10^{-3}	30.3×10^{-3}	2.4×10^{-4}

It would seem that the data in Table 10.15, obtained by combining Henshaw's experiments 1 and 2, show excellent agreement with the concept of a relatively constant α. Although the general rule is doubtlessly more complicated than stated here, it appears that the treatment in Table 10.15 represents at least a first approximation to the principles governing the effect of radiation on survival. Subsequent work may show that intermittency leads to very modified effects and that different results are obtained for exposures terminated after various numbers of exposures. Consequently each roentgen received exercises an effect on survival for radiation that is given chronically throughout the life of an animal, and tolerances can be set only if an acceptable risk is defined. The results on survival are summarized in Table 10.16, which gives the α for several species. These are, therefore, rough estimates of the relative species sensitivities to chronic X radiation and would indicate that the rat, dog, and mouse are approximately equally sensitive to chronic X radiation, the rabbit perhaps more resistant, and the monkey somewhat more susceptible. The correlation with susceptibility to acute doses is only fair since, with single doses of radiation, the order of decreasing susceptibility is dog, C_3H mouse, monkey, rat, and rabbit. It should be borne in mind that acute and chronic susceptibility may be very different.

Table 10.16 — Estimate of Relative Species Sensitivities

Species	α
Rat	2.4×10^{-4}
Dog	2.1×10^{-4}
Rabbit	1.1×10^{-4}
Monkey	5.7×10^{-4}
Mouse (C_3H)	2.0×10^{-4}

3. CLINICAL OBSERVATIONS

The data on peripheral blood are given in Chap. 11. Contrary to expectation, weight data on animals do not provide a very sensitive index of radiation damage. Individual animals customarily maintain their weights rather well until a few weeks before death. Effects of radiation on weight curves are distinctive only at levels above 1 r/day. Figure 10.4 shows the mean weights of all rats by groups throughout the course of the experiment. Figure 10.5 illustrates the uniformity of weight data for rats that survived the 2-year period of radiation. The curves for the selected group are unaffected by changing numbers of animals due to death. Figure 10.6 shows the relative growth rates for intervals of 4 weeks during the first year of radiation. It is obvious that in the rat the tolerance levels of chronic radiation do not affect the relative growth rates.

Corresponding weight curves for dogs, rabbits, and monkeys are shown in Figs. 10.7 to 10.10. The data for monkeys are plotted only for animals that survived at least 200 treatments.

4. EFFECTS ON THE REPRODUCTIVE SYSTEM

Certain observations of interest have been made on sperm production in dogs and are considered here. Male dogs were first examined for sperm about 8 months after the start of chronic radiation. Consequently the preexposure counts on dogs receiving low levels are not available.

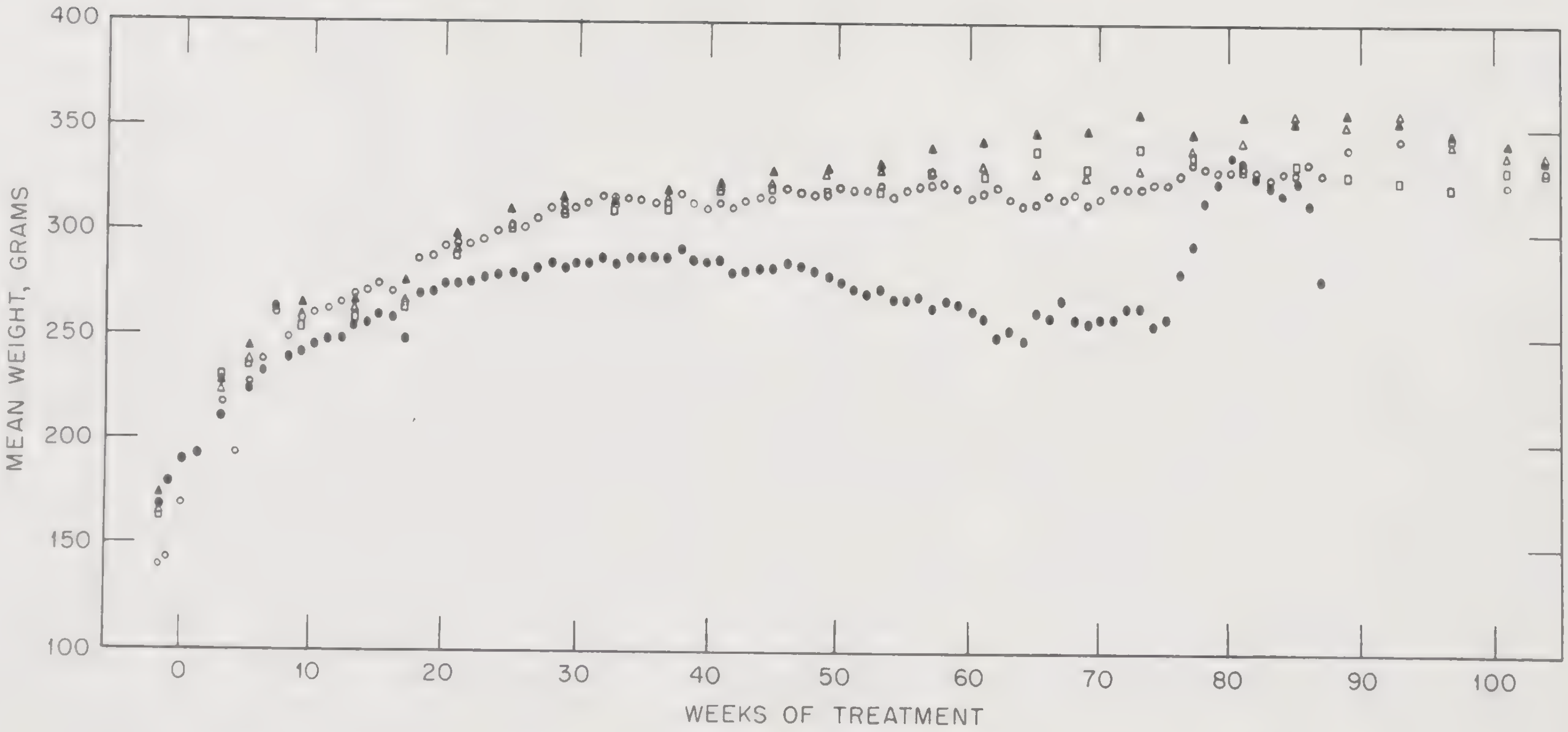

Fig. 10.4—Mean weights of rat groups given chronic exposure to X radiation at various levels. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

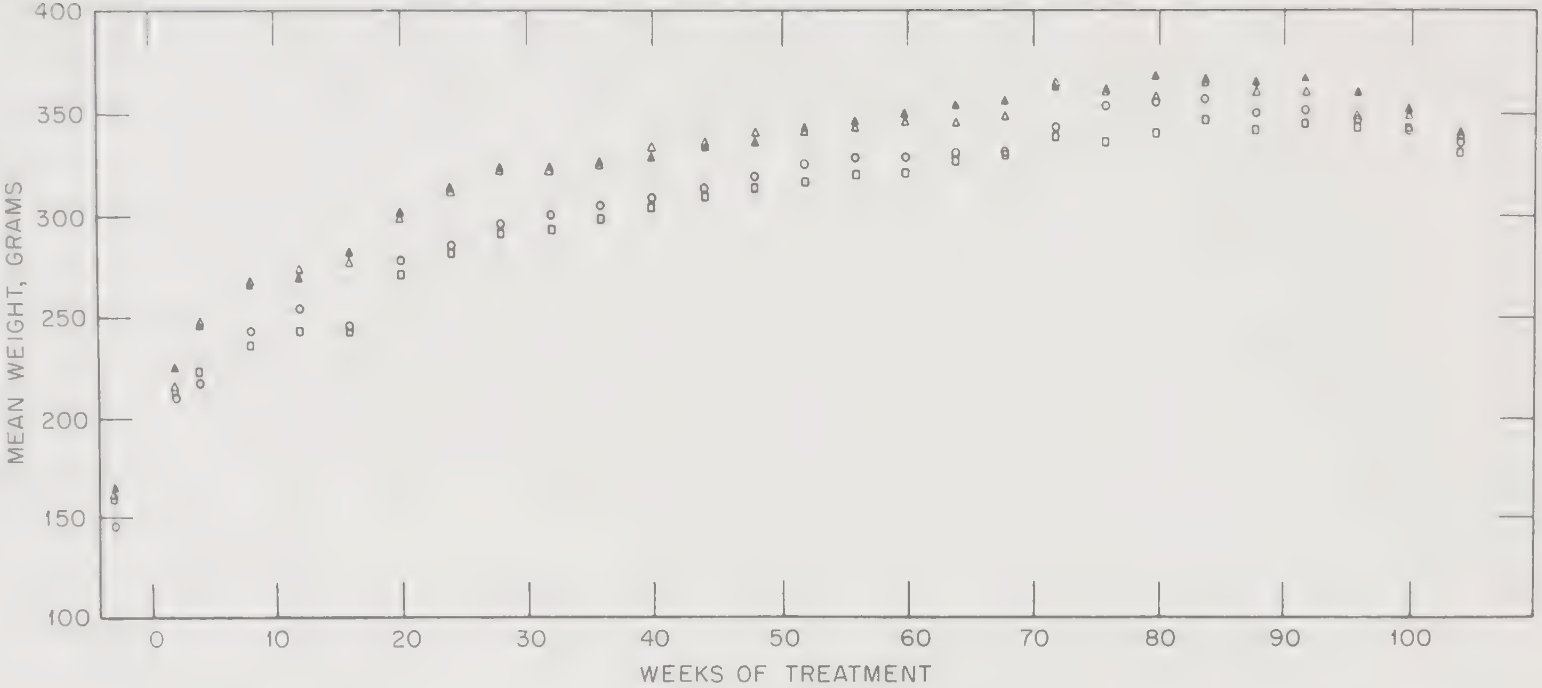

Fig. 10.5—Mean weights of rats that survived the 2-year period of chronic X radiation. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day.

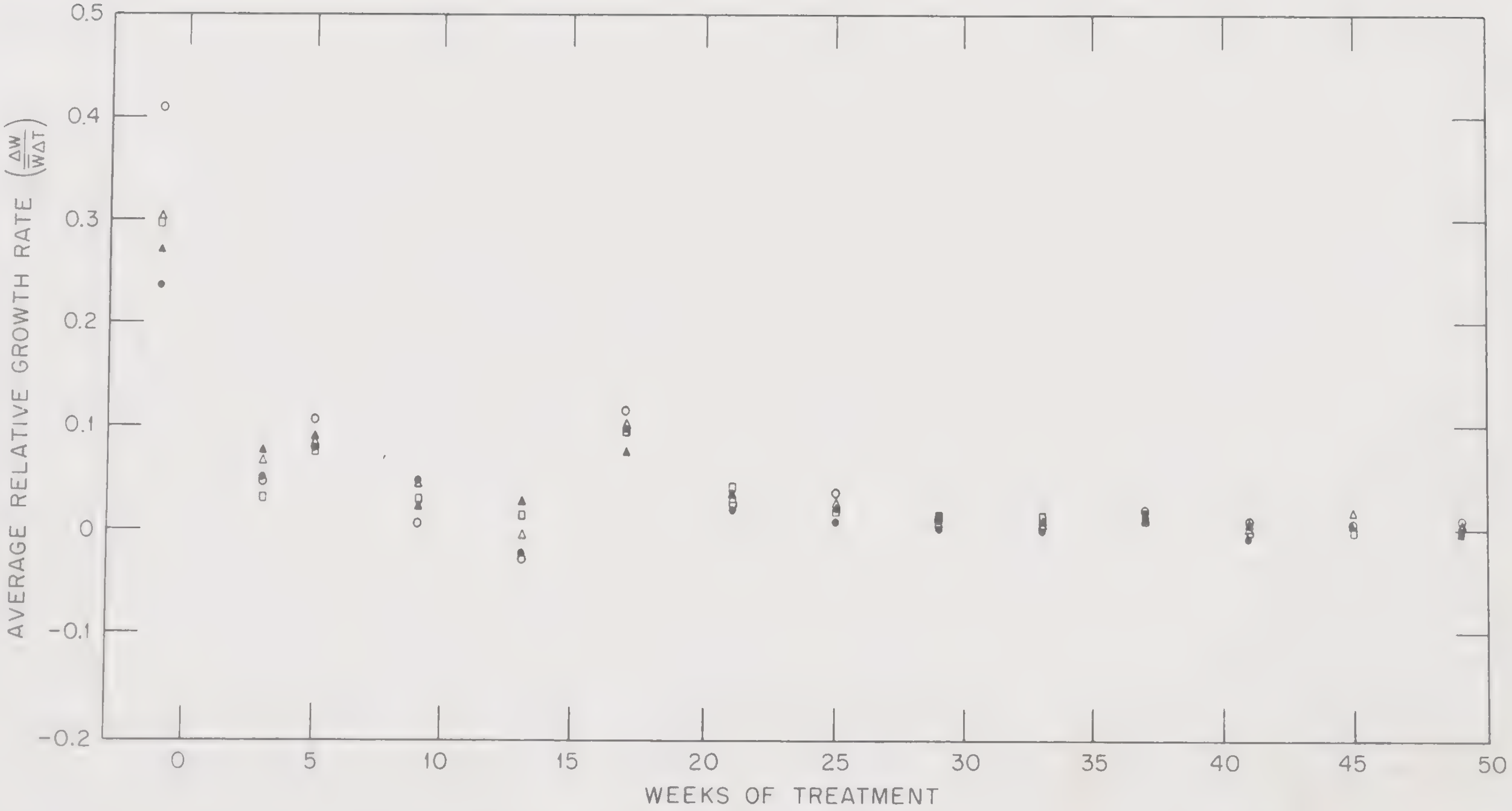

Fig. 10.6—Average relative growth rates of rats during the first year of chronic exposure to X radiation. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

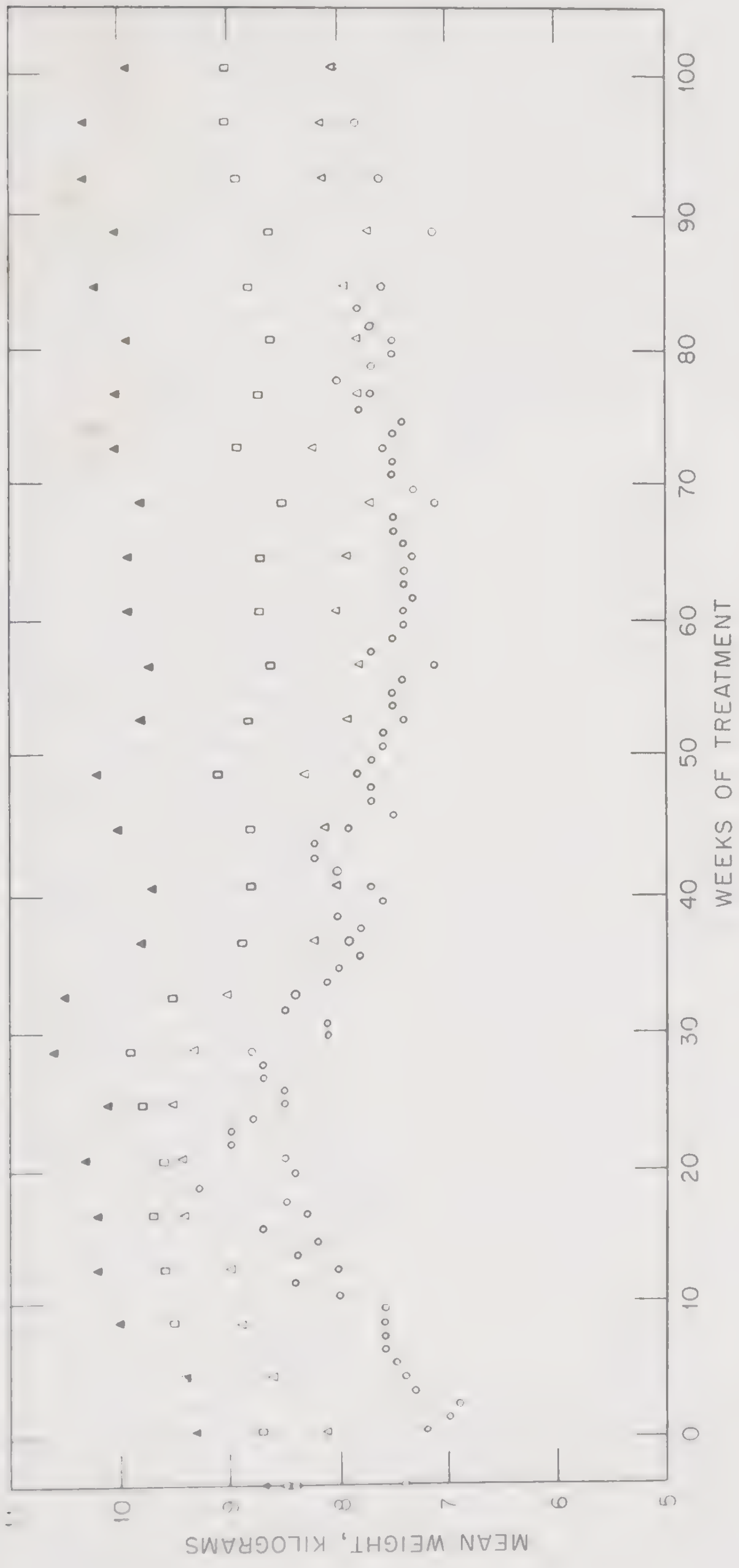

Fig. 10.7 — Mean weights of dogs chronically exposed to X radiation for 2 years. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. Δ, 1.0 r/day.

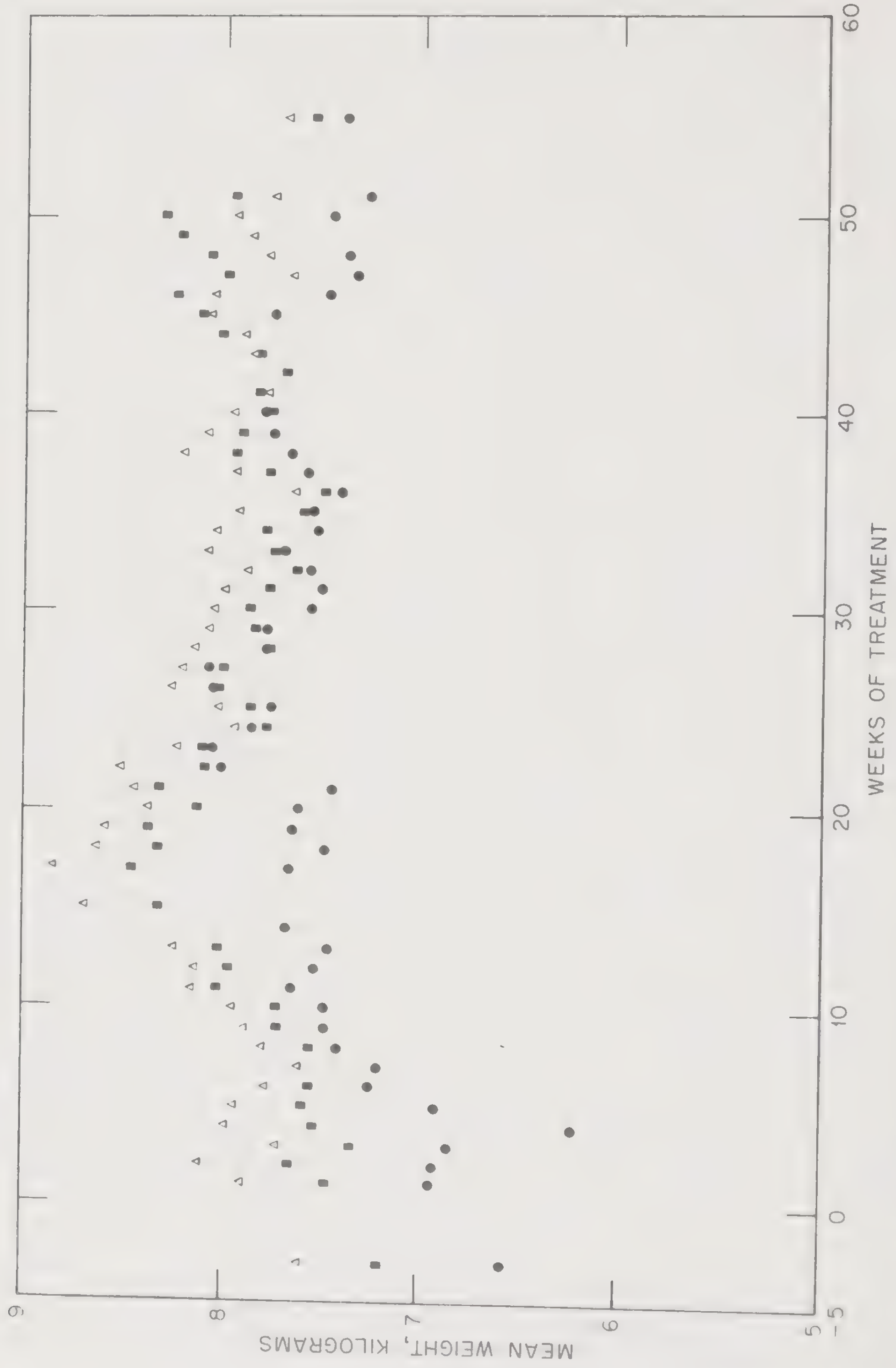

Fig. 10.8—Mean weights of dogs chronically exposed to X radiation. ■, 3.0 r/day. △, 6.0 r/day. ●, 10.0 r/day.

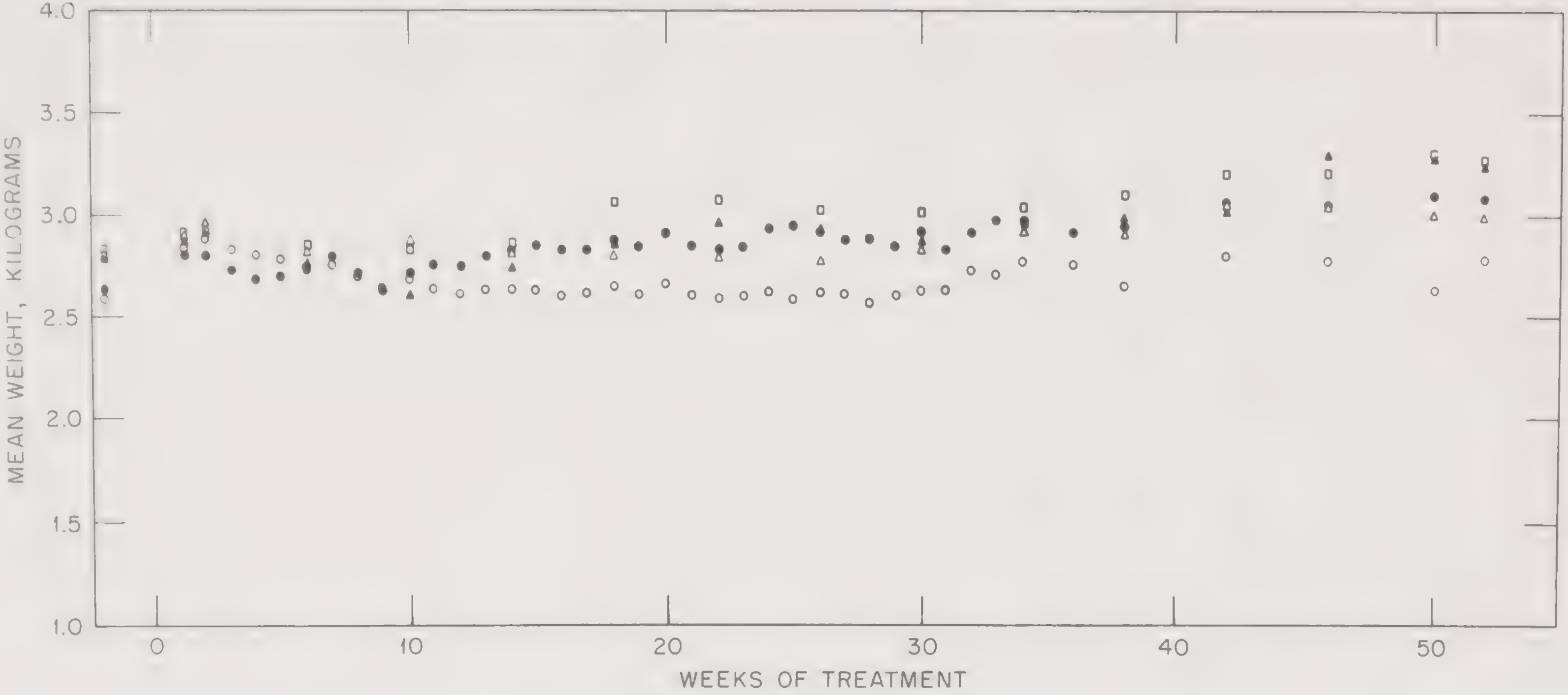

Fig. 10.9—Mean weights by groups of rabbits exposed to chronic X radiation for 1 year. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

Sperm was collected by allowing males to court a female in oestrus after which ejaculation was produced by masturbation. The first examination was a qualitative one using hanging-drop preparations. It was obvious from this that rough quantitative separation of the dogs into three groups was possible: (1) controls and 0.1 r/day dogs, which were indistinguishable from one another; (2) 0.5 r/day dogs, which appeared to have a lowered sperm count; and (3) the 1.0 r/day dogs, about half of which were completely aspermic. Preparations were made to carry out quantitative studies on the sperm count, and this was done at various intervals throughout the remainder of the experiment, including a 5-month postradiation period.

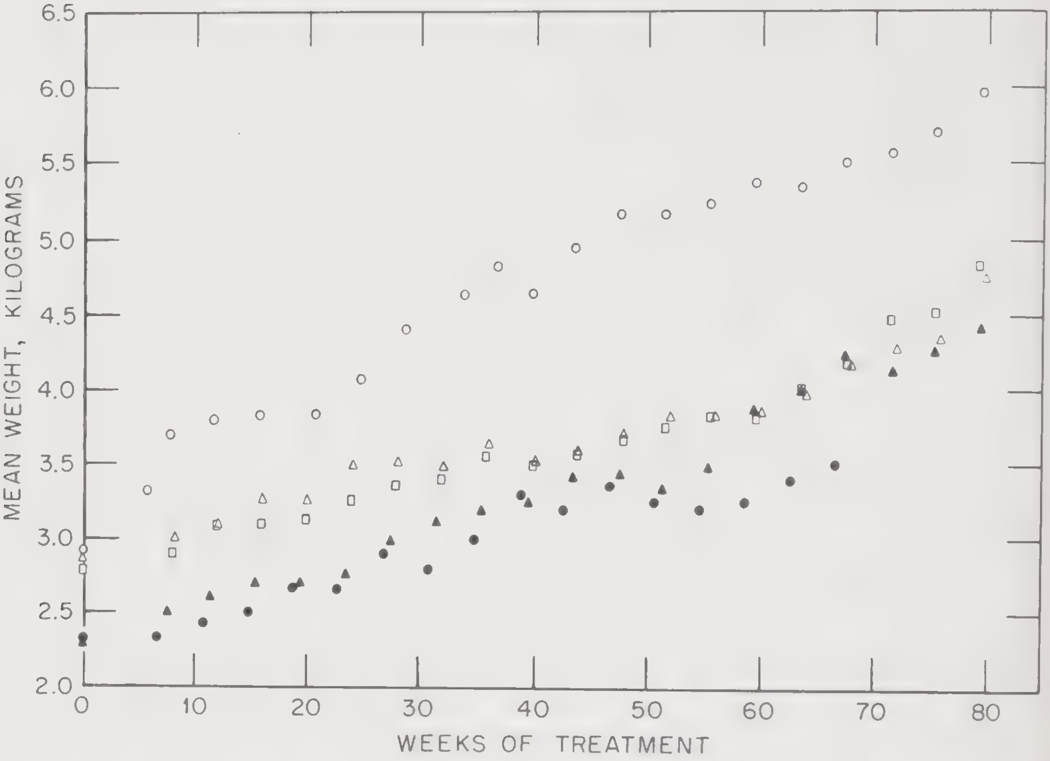

Fig. 10.10—Mean weights of monkeys surviving 200 or more treatments with X radiation. ○, controls. ▲, 0.1 r/day. □, 0.5 r/day. △, 1.0 r/day. ●, 10.0 r/day.

Complete data for each dog are given in Table 10.17. The table shows the volume of semen per ejaculation and the concentration of sperm in millions per cubic centimeter. The variation among groups

Table 10.17 — Volume of Semen* and Concentration of Sperm†
(NS, no specimen obtainable; ND, not done; SAC, sacrificed)

	Controls					0.1 r/Day					
	Dog number					Dog number					
Date	DC1	DC2	DC7	DC8	Date	1DA1	1DA5	1DA8	1DB3	1DB6	1DB10
9/6/44	ND	ND	ND	ND	9/6/44	ND	ND	ND	ND	ND	ND
	+++	++++	NS	++		++++	++++	++	++++	++	+++
1/1/45	ND	ND	ND	ND	1/1/45	ND	ND	ND	ND	ND	ND
	109	53.7	142	221		240	168	29	293	51.4	46
6/5/45	0.6	0.3	0.5	0.7	6/5/45	1.0	0.6	1.1	1.7	0.6	0.5
	27	182	1400	406		388	637	139	221	244	230
9/27/45	0.6	0.7	0.3	0.4	9/27/45	1.2	0.9	0.5	0.6	0.5	0.5
	78	560	380	880		170	51.5	9.2	433	3.2	101
12/18/45	0.5	0.6	0.3	0.3	12/18/45	1.5	1.3	1.3	1.0	0.8	0.75
	264	717	531	945		666	272	0.54	486	0.17	124
12/28/45	0.7	1.2	NS	0.3	12/26/45	1.2	1.7	1.0	0.75	0.8	0.75
	624	354		690		756	342	3	390	0.2	342
1/3/46	0.2	0.4	NS	0.4	1/3/46	0.6	2.0	0.8	1.2	1.2	1.0
	123	456		147		446	297	2.5	297	2.4	237
4/2/46	0.5	0.5	NS	0.3	4/2/46	1.0	SAC	1.5	1.0	0.4	SAC
	0	0.053		1003		394	1/10/46	105	394	364	1/10/46
4/4/46	0.8	0.9	NS	0.4	4/4/46	0.9		0.9	1.5	0.5	
	559	594		76.5		513		16.8	204	602	
4/12/46	0.5	2.0	NS	0.6	4/12/46	0.9		0.7	1.2	0.7	
	9.4	94		237		494		29.4	334	106	
6/10/46	0.3	3.0	NS	0.7	6/10/46	0.3		3.0	NS	0.7	
	65	212		235		65		212		235	
6/12/46	0.6	0.5	0.3	0.7	6/12/46	0.6		1.0	1.5	0.7	
	1092	0	27	101		471		0.025	304	0.025	
6/17/46	0.4	0.3	0.1	0.4	6/17/46	0.7		1.0	1.2	1.0	
	216	0	2.8	142		194		6.0	506	0.025	

*The first line of entries for each date gives the volume of semen in cubic centimeters.

†The second line of entries for each date gives the concentration of sperm in millions per cubic centimeter.

Table 10.17 — (Continued)

	0.5 r/Day						
	Dog number						
Date	2DA2	2DA8	2DA10	2DB3	2DB7	2DB9	2DB10
9/1/44	ND	ND	ND	ND	ND	ND	ND
	++	+++	++	++	++	+++	++
1/1/45	NS	ND	ND	ND	ND	ND	ND
		20	0	4.4	37	0.87	9.0
6/5/45	NS	0.5	0.7	0.7	0.7	1.5	2.5
		115	0	181	153	20.6	0.002
9/27/45	1.0	NS	0.2	0.5	0.6	1.0	1.2
	0.14		1.6	154	92	1.24	0
12/18/45	0.6	NS	0.8	0.5	0.8	0.6	0.9
	0		0	19	22	4.8	0
12/26/45	0.3	NS	0.5	0.8	0.8	1.0	0.7
	4.84		0	119	45	40	0.01
1/2/46	1.0	NS	0.7	1.0	1.0	1.4	0.6
	0.27		0	35	24	18	45
4/2/46	0.7	0.4	SAC	SAC	0.7	1.0	1.5
	200	19	1/10/46	1/10/46	110	15	0
4/4/46	1.3	0.5			0.6	0.6	0.9
	36	90			79.5	1.2	0
4/12/46	1.0	0.5			1.1	1.5	1.2
	62	5.2			38	101	4.5
6/10/46	1.4	0.3			0.7	1.4	1.3
	68	299			180	357	81.4
6/12/46	1.3	0.3			1.0	0.8	1.0
	103	502			349	23	102
6/17/46	1.0	0.5			0.8	1.1	1.0
	18	348			171	86.6	13.8

	1.0 r/Day									
	Dog number									
Date	3DA3	3DA4	3DA6	3DB1	3DB2	3DB3	3DB4	3DB8	3DB9	3DB10
9/1/44	ND	ND	ND	NS	ND	ND	ND	ND	ND	ND
	0	0	0		+	0	+	+	+	+
1/1/45	ND	ND	ND	NS	ND	ND	ND	ND	NS	ND
	0	0	0		0	0	0	0		0
6/5/45	0.4	0.3	0.8	NS	1.3	0.1	0.7	0.6	NS	1.2
	1.7	0	0		0	0	0	0	0	0.002
10/3/45	Dead	0.7	0.3	NS	1.4	1.8	0.5	0.8	Dead	1.5
		0	0		0	0	0	0		0
12/18/45	Dead	ND	ND	ND	ND	ND	ND	ND	Dead	ND
12/26/45	Dead	ND	ND	ND	ND	ND	ND	ND	Dead	ND
1/2/46	Dead	ND	ND	ND	ND	ND	ND	ND	Dead	ND
4/2/46		SAC	0.7	1.0	SAC	1.0	1.4	0.9		SAC
		1/10/46	460.08	0.53	1/10/46	0	0	0		1/11/46
4/4/46			0.3	0.6		0.9	1.2	0.9		
			1.9	0.12		0	0	0		
4/12/46			0.4	0.6		1.1	1.3	1.0		
			0.63	26.8		0	0	0		
6/10/46			0.6	NS		0.4	0.9	0.8		
			187			0	0	16		
6/12/46			0.2	NS		1.0	1.4	0.8		
			0.025			0	0	40.4		
6/17/46			0.3	0.4		0.5	0.6	0.7		
			0	203		0	0	28		

is obvious, as is the fact that occasionally a dog will have zero sperm count on one day and an enormous count a week later or vice versa. The explanation for such erratic behavior is not clear but may lie in the artificial conditions created for collecting the samples. In any case, aspermic dogs are largely confined to the 1.0 r/day group, half of which were aspermic at 9 months after the start of radiation, the remainder becoming so in the following 3 months. At one time, about half the 0.5 r/day dogs were aspermic, although the chief finding in this group was a lowered sperm count. Since it was a major objective of this experiment to discover whether the so-called "tolerance levels" produced any detectable biological effects, further analysis of the sperm counts of controls as opposed to 0.1-r dogs is given.

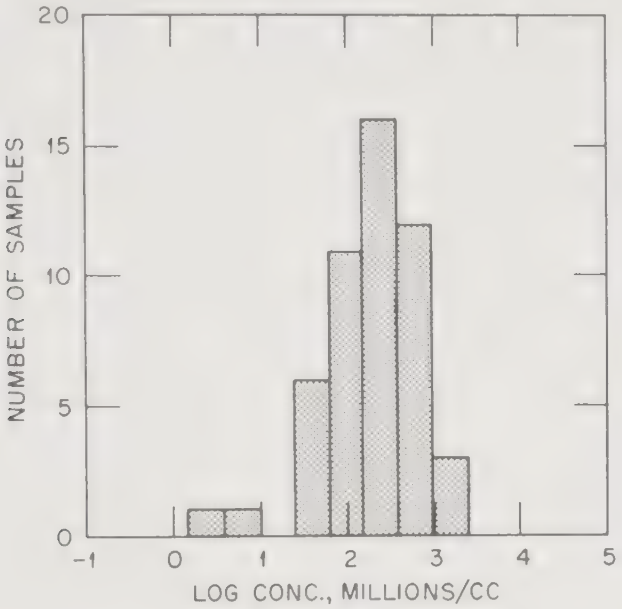

Fig. 10.11 — Frequency distribution of logarithm concentration for sperm from all controls and 0.1-r dogs at the first two counts.

If aspermic dogs were considered as belonging to a different distribution and therefore were neglected, it was found that frequencies of sperm counts of different magnitudes may be normalized by the transformation to logarithms.[3] Figure 10.11 shows the distribution of log count for all controls combined with the first two quantitative counts on 0.1-r dogs. Near the end of the period of radiation, three successive counts were made on all dogs (except those at 1.0 r/day which were aspermic), and these were repeated in triplicate 3 and

5 months postradiation. Statistical analysis shows that the effects at 0.1 r/day are probably real. The average logarithms of counts (log geometric mean) have been plotted and are shown in Fig. 10.12. Attention is directed to the fact that, in the second year of radiation, 0.1 r/day dogs deviate from controls and 0.5 r/day dogs are much lower, whereas 1.0 r/day dogs have zero count, and that, in the postradiation interval, there is evidence of recovery in all treated groups, including 1.0 r/day dogs, about half of which have resumed the production of motile sperm. The volumes of semen per sample are apparently quite unaffected by exposure to radiation at the levels studied, i.e., variations are randomly distributed. Hence it is immaterial whether total sperm per ejaculation or sperm concentrations are studied.

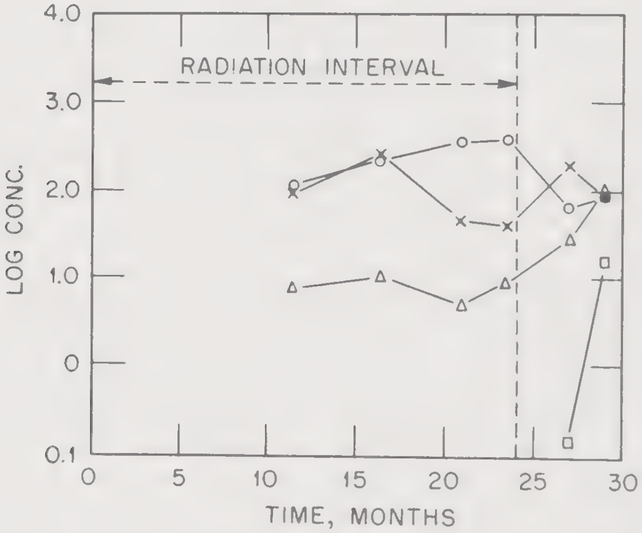

Fig. 10.12—Average logarithms of sperm counts in dogs exposed to chronic X radiation. ○, controls. ×, 0.1 r/day. △, 0.5 r/day. □, 1.0 r/day.

In addition to hemacytometer counts of sperm concentration, differential counts of abnormal forms in stained smears were made. In practice the number of sperm having two heads, two tails, curled tail, or an enlarged midpiece were recorded for a total of 200 sperms per sample. These data have been converted to percentages of abnormal forms for each animal and averaged by groups. All such data for control, 0.1-, and 0.5-r dogs are included in the averages shown in Fig. 10.13. It would be difficult to believe that the observed reductions in sperm count, especially when corrected for percentages of abnormal forms and in view of subsequent recovery, are not real effects of radiation.

A few data have been obtained on sperm counts of dogs at higher radiation levels. Approximately half the dogs on which sperm counts were available at the start of exposure were rendered aspermic by 3, 6, or 10 r/day in 4 months (100 treatments), and four out of five dogs became aspermic in 9 months at 3 r/day and five out of six in 9 months at 6 r/day. The two dogs with sperm at these levels had counts of 40,000/cc. Two animals at the 3-r level, three at the 6-r level, and two at the 10-r level were examined at the end of 500 treatments and found to be aspermic in agreement with expectation. Higher dose levels are apparently somewhat less efficient per roentgen than low levels, a reasonable interval of time being required for the effect to develop even at 10 r/day. This may, in part, be due to the fact that mature sperm and perhaps the later stages in spermatogenesis are insensitive to radiation, and these forms may persist for a time.

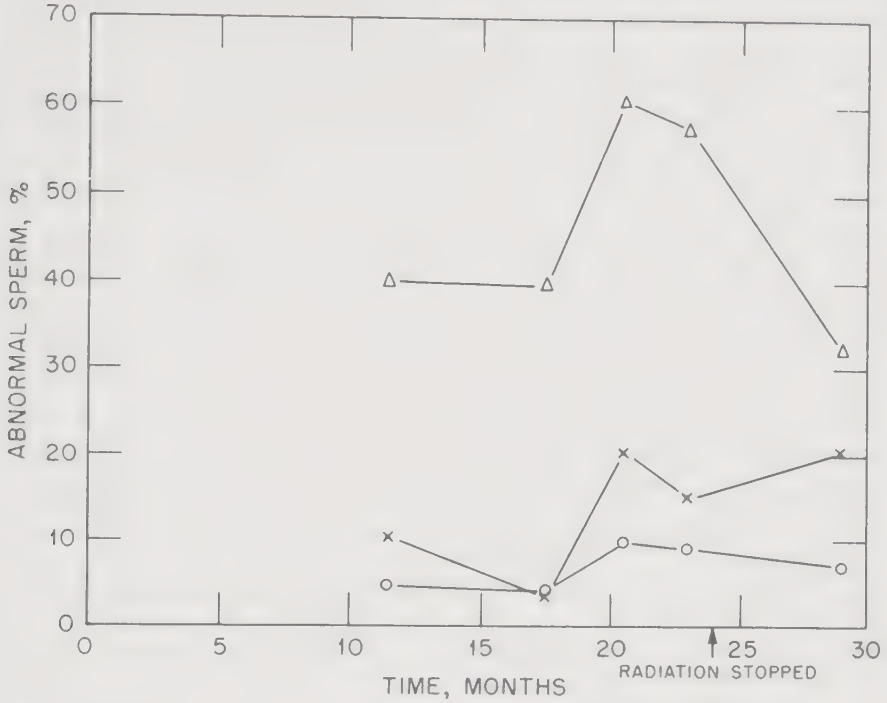

Fig. 10.13—Average percentages of abnormal sperm in dogs subjected to chronic X radiation for 2 years. ○, controls. ×, 0.1 r/day. Δ, 0.5 r/day.

These effects on the reproductive system of dogs are apparently confined to the male because several accidental pregnancies occurred, one of which involved a control male dog and a female who had received approximately 440 treatments at 3 r/day. She carried her litter for a normal period of gestation, during which radiation was continued, and produced six pups, all normal.

No instructive data on reproductive effects are available for rabbits or monkeys, although a single pair of monkeys receiving 0.1 r/day were parents of a healthy male conceived at a time when both parents had been given approximately 300 treatments or 30 r. The mother was irradiated during the entire period of gestation (5½ months), and the young monkey thus received about 15 r during fetal life. It was raised to the age of 13½ months and was then sacrificed. Male rats receiving 1 r/day for 2 years were still producing motile sperm at the end of the radiation period. However, quantitative data on the rat could not be obtained because of the solid character of the rat semen.

The mechanism by which sperm production is inhibited is unknown, and it has not been possible to analyze the process further. Several possibilities suggest themselves, such as disturbances at meiosis or effects on the cell spindle or centrioles. The general depletion in radiated testes of all types of germinal elements suggests that the block occurs early in spermatogenesis rather than in the later stages. Part of the sensitivity of the testes may reside in the possible lack of compensatory mechanisms of the type believed associated with depressions in circulatory red or white blood cell counts. In any case the problem merits further study in itself and as material for providing additional information on the mechanism of tissue depletion by radiation.

A variety of tumors were observed in rats and a few in dogs. Since they are discussed in detail by kind in Chap. 12, it is sufficient to say here that the majority of benign mammary fibroadenomas and nearly all the leukemias were attributable to radiation. Malignancy as a hazard in radiation exposure is also discussed in Chap. 12.

ACKNOWLEDGMENTS

The author is greatly indebted to M. J. Wantman, D. V. Tiedeman, and the staff of the Statistics Division for assistance with the computation of mean weights and expresses his appreciation to T. B. Barnett, J. S. Youngner, Marjorie Goring, and Mary E. Gould for their assistance in obtaining data on sperm production.

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Chapter 11*

EFFECTS OF CHRONIC EXPOSURE TO X RADIATION ON THE PERIPHERAL BLOOD OF EXPERIMENTAL ANIMALS

By M. Ingram and W. B. Mason

These studies on the hematological effects of chronic exposure to X radiation were carried out from 1944 to 1946 under the direction of F. A. Bryan and G. M. Suter and were designed to supply information relative to both the nature and the magnitude of the changes which follow repeated exposure to radiation in amounts near the tolerance range. The urgency of the problem in supplying information essential to the well being of the many persons potentially exposed to radiation has already been indicated by A. H. Dowdy et al. in Chap. 9 and requires no further elaboration. As in the case of the acute-radiation study (Chap. 3), the work described in this chapter was designed with emphasis on those hematological effects of radiation which seemed most suitable for translation into a practical monitoring system which would utilize changes in the worker as an indication of his radiation exposure.

1. METHODS

The hematological observations represent a part of a group of studies which also encompass pathology, survival, and blood chemistry. In many instances all these investigations were carried out on the same groups of animals. Dogs, rabbits, rats, and monkeys were used in the hematological investigation. The monkeys and rats, however, receive only casual mention throughout this chapter since they acquired considerable extraneous pathology and there is little or no justification for attributing any of the observed changes to the radiation received.

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* Based on Report UR-121, July 17, 1950.

Details of the protocol, animal selection and care, and radiation techniques are presented in Chap. 9 and will not be restated here. In general, the experiments were designed so that, for each group of animals receiving a given dosage from the 250-kvp machine, a comparable group received the same dosage from the 1000-kvp machine. This permitted a comparison of the effects of like quantities of whole-body radiation from the two sources. The dosages administered were 0.1, 0.5, 1.0, and 10.0 r/day, respectively, for the four species. In the case of dogs, there were two additional groups, one receiving 3.0 r/day and the other, 6.0 r/day. With the exception of the rabbits and the 3.0 and 6.0 r/day dogs, all of which were irradiated for a maximum of only 1 year, the indicated dosages were administered daily, 6 days a week, until the animals died or until a total of 2 years had elapsed. Blood counts were made at regular intervals.

Blood sampling and blood counts on dogs, rats, and monkeys were done as in the acute studies (Chap. 3). Rabbits were bled from the marginal ear vein either by venepuncture or by cutting across the vein with a Bard-Parker blade, and all counts were made on the freely flowing blood. All dogs had two blood counts before receiving radiation. Rats and rabbits had but one count each before the beginning of treatment. Enumeration of the red blood cells, white cells, platelets, and reticulocytes; hemoglobin determination; differential leucocyte count; and an evaluation of basophilia, stippling, anisocytosis, and poikilocytosis comprised a complete blood count. Differential leucocyte counts were made by classifying 100 cells on a Wright's stained blood smear.

Table 11.1 presents the protocol of the chronic X-ray experiments.

2. FACTORS INFLUENCING INTERPRETATION OF RESULTS

One of the most difficult aspects of experimental studies involving any type of living organism concerns the evaluation of the normal limits of variation of the property selected for study. Of no less importance is the recognition of extraneous factors and technical procedures which may influence the experimental findings. These matters are especially important in long-term studies in which the experimentally induced changes are expected to be minimal. For this reason certain observations made during the course of these experiments are discussed briefly in the following paragraphs.

From the start the monkeys seemed poorly suited to the experiments. Not only were they difficult to handle, but they acquired considerable extraneous pathology, chiefly infections of the respiratory tract, and it was finally necessary to exclude entirely the results based on these animals.

It will be noted that the duration of the experimental observations encompasses a large part of the normal life span of rats. This introduces an undesirable variable which can be, at best, only partly compensated for by the use of control groups. A much more disturbing problem in the rat experiments concerned the severe leucocytoses which developed in some individuals in all groups. In many instances the leucocytoses were transient, but in others they were persistent or occurred shortly before the animal died. Almost always they were accompanied by a marked increase in the percentage of polymorphonuclear neutrophils. A very small percentage of the hemograms were definitely leukemic with many immature forms in the smears of the peripheral blood. More often, however, the leucocyte counts were in

Table 11.1 — Protocol of the Chronic X-ray Experiments

Species	Daily dosage,* r	Duration of experiment, years	Frequency of blood counts
Dogs	0.0†	2	Monthly
	0.1	2	Bimonthly
	0.5	2	Bimonthly
	1.0	2	Bimonthly
	3.0	1	Weekly
	6.0	1	Weekly
	10.0	1	Weekly
Rabbits	0.0†	1	Monthly
	0.1	1	Bimonthly
	0.5	1	Bimonthly
	1.0	1	Monthly
	10.0	1	Weekly
Rats	0.0†	2	Bimonthly
	0.1	2	Bimonthly
	0.5	2	Bimonthly
	1.0	2	Bimonthly
	10.0	2	Biweekly
Monkeys	0.0†	2	Monthly
	0.1	2	Bimonthly
	0.5	2	Bimonthly
	1.0	2	Bimonthly
	10.0	2	Bimonthly

* Dosage administered daily, 6 days a week; each dosage administered to two groups of animals, one receiving radiation from a 250-kvp machine, the other group from a 1000-kvp machine.

† Controls.

the range of 30,000 to 50,000 cells/cu mm, with 50 to 90 per cent neutrophils and some shift to the left but with very few or no abnormal cells. In almost all cases these leucocytoses appear to have been related to severe infection, which occurred frequently in the tails. This was often advanced enough to produce sloughing. Sampling blood from the tails of such rats presents a problem, even when the blood is taken from a point as far proximal to the inflammation as possible.

There were other serious infections noted among the rats (see Chap. 12), and these add to the difficulty in interpreting results. Although severe leucocytoses might be identified, it is impossible to detect mild cases which would tend to mask radiation-induced leucocyte depressions of small magnitude. Selection of data in such a situation is extremely hazardous, and, inasmuch as the observed changes in many instances were very small and could be demonstrated only by statistical methods, the findings in the chronic X-ray studies on rats have been excluded from this chapter.

Differential leucocyte counts during stages of severe leucopenia were occasionally based on the classification of fewer than 50 cells. This technique was used less often in these studies, in which many of the leucocyte depressions were slight, than in the acute experiments (Chap. 3). In the cases in these experiments in which marked depression did occur, however, the uncertainty introduced by classifying only a limited number of cells is significant.

The protocols for the early experiments prescribed blood counts at definite intervals during the period of irradiation, with the counts on individual animals within each experimental group staggered in such a manner that the composite data would provide a detailed picture of the changes which occurred. As the experiments progressed, however, it became apparent that the experimental changes were obscured by normal fluctuations. It thus became necessary to examine the data statistically. This, in turn, necessitated grouping the counts according to arbitrary intervals. For example, the count 1 month after the beginning of the experiment was determined by calculating the mean of all counts made at any time during the first month. This most unsatisfactory procedure was corrected before the rabbit experiments and the 3.0 and 6.0 r/day dog experiments were begun, and in these experiments all the animals composing a given group had their blood counts made at the same time.

Repeated preexposure blood counts were made on certain of the early experimental groups. The values obtained, however, were essentially constant, and later experimental groups usually had but one preexposure count. When it became necessary to treat the data by statistical methods, however, it was readily apparent that a large series of preexposure counts would have facilitated interpretation of the results by providing valuable information relative to the similarity or dissimilarity of the control and experimental groups. The value of repeated preexposure blood counts was unfortunately not appreciated until after the experiments were completed, and this factor alone is responsible for considerable uncertainty in interpreting much of the data.

3. RESULTS

In the interpretation of the results from the chronic experiment, particular attention has been given to the statistical evaluation of the changes which were observed. The analysis-of-variance technique was applied to the data, a trend being tested first against the variation around its component points and finally against a control experiment. The probability of the chance occurrence of a change as great as, or greater than, the one observed is expressed by a value p. A value of 0.10, for example, indicates that a deviation as large as the one under consideration would be expected to occur by chance in 10 per cent of the cases. Values of p of 0.05 or below are considered to be statistically significant, whereas values of less than 0.01 are considered to be highly statistically significant.

It was not feasible to test all points in each experiment for the significance of their variation from normal. For the most part, only those points representing the 1- and the 2-year intervals were tested. The hematological effects observed in dogs and rabbits after 1 and 2 years of daily exposure to small doses of **X** radiation are summarized in Table 11.2.

Mortality in the experimental groups was considerably lower in these experiments than in the acute studies (Chap. 3). A detailed study of survival data is presented in Chap. 10, where it may be noted that only in the three higher-dosage groups was mortality sufficiently high to be of importance in the interpretation of results. In conformance with the practice adopted in Chap. 3, the trends of various cellular elements of the peripheral blood described in the following sections are, in all cases, based on the findings in all animals surviving at the time under consideration.

3.1 Effects on the Peripheral Blood of Dogs. (a) Lymphocytes. Lymphocyte counts underwent no significant change during 2 years of irradiation with 0.1 r/day. Lymphocytes of all dogs exposed to doses of 0.5 r/day and above, however, were decreased significantly. Depressions developed abruptly after the beginning of irradiation in the

Table 11.2—Statistically Significant Trends of Cellular Elements of the Peripheral Blood Following Chronic Exposure to Radiation*

Species	Dose, r/day	Absolute lymphocytes after exposure for				Absolute neutrophils after exposure for				Total leucocytes after exposure for				Erythrocytes after exposure for			
		1 year		2 years		1 year		2 years		1 year		2 years		1 year		2 years	
		250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv
Dogs	0.1	0	0	0	0	0	0	0	0	0	0	0	0	□	□	0	0
	0.5	0	0	– 0.01 – 0.05	– 0.01 – 0.05	0	0	0	0	0	0	0	0	□	□	0	0
	1.0	0	0	– 0.01 – 0.05	0	– 0.01 – 0.05	– < 0.01	0	– < 0.01	– 0.01 – 0.05	– < 0.01	– 0.01 – 0.05	– < 0.01	□	□	0	0
	3.0	– < 0.01	– < 0.01	Δ	Δ	0	– 0.01 – 0.05	Δ	Δ	– 0.01 – 0.05	– < 0.01	Δ	Δ	0	+ < 0.01	Δ	Δ
	6.0	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	– 0.01 – 0.05	0	Δ	Δ
	10.0	– < 0.01	– 0.01 – 0.05	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ
Rabbits	0.1	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ
	0.5	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ
	1.0	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ	0	0	Δ	Δ
	10.0	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	– < 0.01	– < 0.01	Δ	Δ	0	– < 0.01	Δ	Δ

* 0, no significant trend (p greater than 0.05). +, significant increase (numbers equal p value). –, significant decrease (numbers equal p value). □, not analyzed statistically; no apparent change. Δ, experiment not continued longer than 1 year.

3.0, 6.0, and 10.0 r/day groups. In these groups lymphopenia was marked 1 week after the beginning of irradiation and was sustained throughout the remainder of the experiment. Depressions developed more slowly and were less marked in the 0.5 and 1.0 r/day dogs in which lymphopenia appears not to have been definite until approximately the sixth month of irradiation. Characteristic lymphocyte trends are presented graphically in Fig. 11.1.

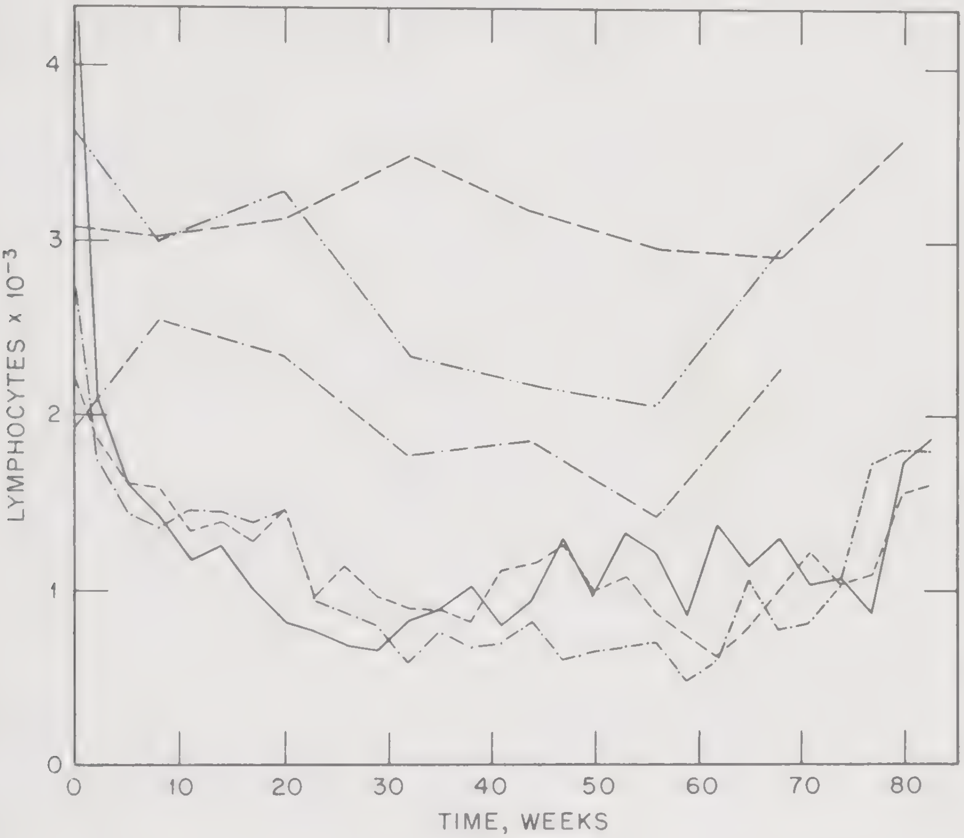

Fig. 11.1—Lymphocyte trends in dogs exposed daily to X radiation. ——, 10 r/day. -·-, 6 r/day. ----, 3 r/day. -··-, 1 r/day. ·--·, 0.5 r/day. — —, controls.

Depressions were statistically significant after 2 years of exposure to 0.5 r/day from both machines and after the same duration of exposure to 1.0 r/day from the 250-kvp machine. Neither of these groups showed statistically significant depressions at the end of the first year of exposure. Lymphocytes of the three higher-dosage groups, however, showed statistically significant depressions when tested at the 1-year interval.

(b) Neutrophils. Neutrophil trends were highly erratic and for the most part were not apparent by inspection. There was no statistically significant reduction of neutrophil counts at either the 1- or 2-year interval in the 0.1 or 0.5 r/day groups. However, there was a significant depression after 1 year of irradiation with 1.0 r/day from both machines. The statistically significant depression was still demonstrable at the end of the second year of the same exposure from the 1000-kvp machine only.

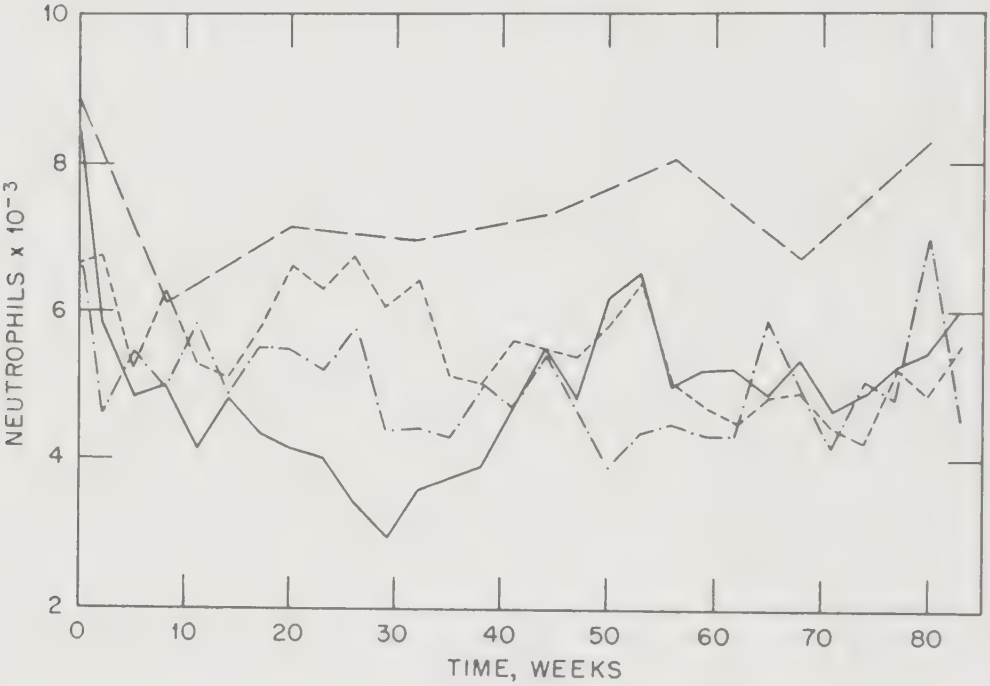

Fig. 11.2—Neutrophil trends in dogs exposed daily to X radiation. ——, 10 r/day. —·—, 6 r/day. ----, 3 r/day. — —, controls.

Absolute neutrophil counts of both 3.0 r/day groups were extremely irregular but appeared to be somewhat decreased at the end of the year of exposure. This decrease was statistically significant only in the 1000-kvp group.

Neutrophil counts of the 6.0 and 10.0 r/day groups also varied markedly, but the over-all trends were definitely downward as early as 1 month after the beginning of exposure, and the depressions at the end of 1 year of exposure in these groups were highly statistically significant. The neutrophil trends in the 1000-kvp 3.0, 6.0, and 10.0 r/day dosage groups are presented graphically in Fig. 11.2.

(c) Total Leucocytes. Trends of the total leucocyte counts represent a composite of the trends of the neutrophils and lymphocytes. Depression of the total leucocyte counts was effected within 2 months after the beginning of irradiation in the 1.0, 3.0, 6.0, and 10.0 r/day groups. The initial decreases were small in the 1.0 and 3.0 r/day groups but were precipitous in those animals receiving 6.0 and 10.0 r/day. Trends continued irregularly downward in all these groups and were roughly proportional to the dosage. Owing to the variation between successive counts within a given dosage group, however, the stratification of counts was not entirely consistent.

The reduction of total leucocytes in the 1.0 and 3.0 r/day groups irradiated from the 1000-kvp machine appeared to be somewhat more pronounced than the reductions in the same dosage groups irradiated from the 250-kvp machine. The data are such, however, that no significance can be attached to this finding.

There was no reduction of the total leucocyte counts after 2 years of exposure to 0.1 and 0.5 r/day from either machine.

(d) Platelets. Platelet counts were depressed in all dogs treated with 3.0, 6.0, and 10.0 r/day from either the 250- or the 1000-kvp machine. The depressions appear to have been abrupt within the first 2 months and slowly progressive thereafter. In the 3.0 r/day group the apparent depression is not confirmed by statistical analysis. The 0.1, 0.5, and 1.0 r/day groups showed no definite depression of the platelet counts. Because of the marked variation in the platelet counts in all groups, however, only the very prominent depressions are apparent either by inspection or by statistical analysis.

(e) Erythrocytes. The only treatment group which developed a definite decrease in erythrocytes following daily irradiation was the 10.0 r/day group. The depression of erythrocytes in this group was marked. It is difficult to describe the depression in detail, however, because the two subgroups of five animals each which were utilized in the experiment were irradiated at different seasons, and, although both subgroups developed anemia, they differed considerably in the details of their erythrocyte trends and in their mortality.

No significance is attached to the variation in erythrocyte counts of the 3.0 and 6.0 r/day groups.

The lower-dosage groups and the controls showed a slight increase in the red blood cell counts during the period of the experiment. This, of course, cannot be attributed to irradiation. Hemoglobin trends were closely similar to erythrocyte trends in all cases.

Erythrocyte trends of the controls and of the three higher-dosage groups are presented graphically in Fig. 11.3.

(f) Reticulocytes. There was extreme variation in the reticulocyte counts of all animals, including the controls, throughout the experiment. The counts of all groups also tended to fall progressively throughout the 1- or 2-year period. There was no apparent significant difference between the trends of the three lower-dosage groups and the controls. The 3.0, 6.0, and 10.0 r/day groups, however, all showed apparently definite reticulocyte depressions of approximately equal magnitude at the time of the first postexposure count, i.e., 1 week following the beginning of irradiation. This depression continued throughout the period of irradiation. The marked reticulocytosis which characterized the recovery response to acute exposure to larger doses was not observed.

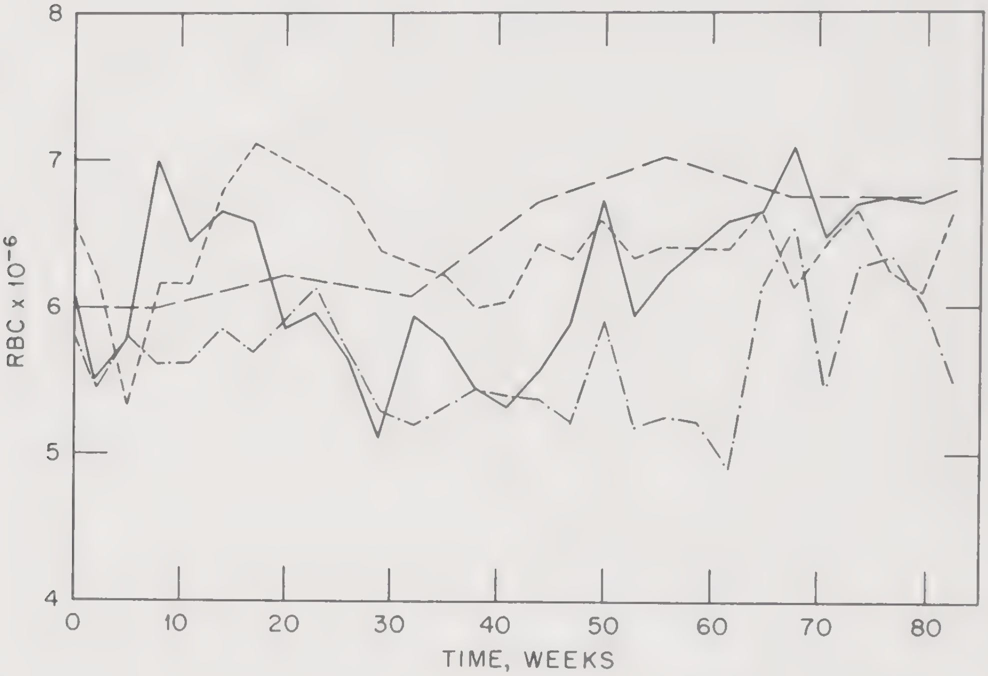

Fig. 11.3—Erythrocyte trends in dogs exposed daily to X radiation. ——, 10 r/day. —·—, 6 r/day. ----, 3 r/day. — —, controls.

3.2 Effects on the Peripheral Blood of Rabbits. The only rabbits which developed significant changes in any of the cellular elements of the circulating blood were those which received 10.0 r/day. This contrasts sharply with the findings in dogs and rats, where prominent changes were noted with amounts as small as 0.5 r/day. The difference in response between these animals serves to demonstrate the species variation which has long been known and indicates one of the

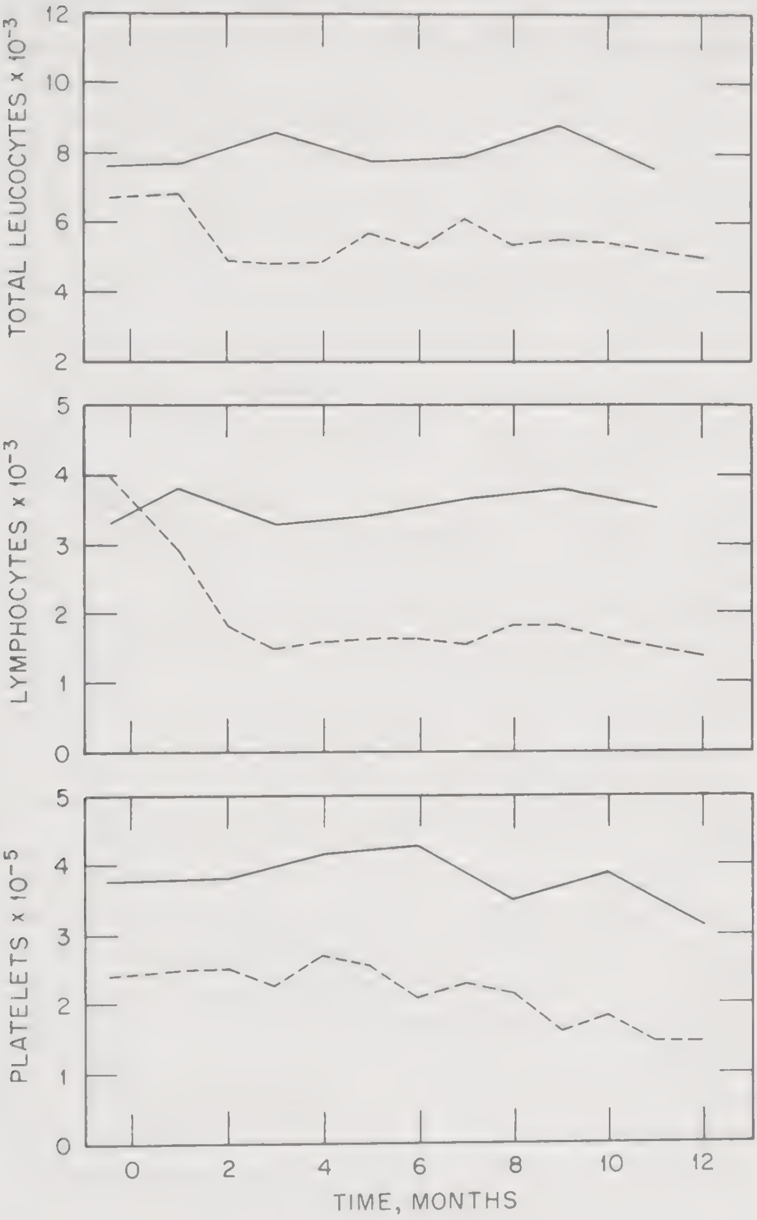

Fig. 11.4 — Lymphocyte, total leucocyte, and platelet trends in rabbits exposed daily to X radiation. ——, controls. ----, 10 r/day. 250-kvp machine.

dangers inherent in applying data obtained in animal experiments to human beings. The trends of lymphocytes, total leucocytes, and platelets for those animals receiving 10.0 r/day are presented graphically in Fig. 11.4.

(a) Lymphocytes. Absolute lymphocytes decreased rapidly after the beginning of exposure and were significantly depressed after 8 weeks of irradiation at the rate of 10.0 r/day. The count then stabilized and remained relatively constant with minimal fluctuations throughout the remainder of the experiment. There was no demonstrable difference in the effects of irradiation from the 250- and the 1000-kvp machines.

(b) Neutrophils. Neutrophil depressions were minimal in rabbits exposed to 10.0 r/day, and the depression was statistically significant only in the group which was irradiated from the 250-kvp machine. The slight depression which did occur appeared to be definite approximately 5 weeks after the beginning of exposure. The level reached at that time was maintained with only minor variations throughout the remainder of the experiments.

(c) Total Leucocytes. A statistically significant depression of the total leucocyte counts in rabbits was present after approximately 1 month of exposure to 10.0 r/day from both 250- and 1000-kvp machines. The counts tended to stabilize at this level and varied little thereafter. The trends and the magnitude of depression were approximately the same in the two groups.

(d) Erythrocytes. A statistically significant depression of the erythrocyte count of rabbits was demonstrated after 1 year of exposure to 10.0 r/day from the 1000-kvp machine. The p value for this trend lies between 0.01 and 0.05. There was also an apparently significant decrease in erythrocytes of the 10.0 r/day group which received radiation from the 250-kvp machine, and the depression appeared to be of approximately the same magnitude as that described in the 1000-kvp 10.0 r/day group. There was greater variation in the counts of rabbits irradiated from the 250-kvp machine, however, and the p value for the erythrocyte depression in this group is just greater than 0.05. The trend therefore cannot be considered statistically significant.

The depressions, although small, appeared to be real after approximately 4 months of treatment and to progress very little thereafter. However, owing to the variations in all groups, including controls, variations of small magnitude, such as the ones described above, must be evaluated with caution.

(e) Platelets. Platelets were definitely reduced by the third month after the beginning of irradiation at the rate of 10.0 r/day. Both the 250- and the 1000-kvp machines produced this effect. The platelet depression, once established, persisted and varied little throughout the remainder of the experiment.

(f) Reticulocytes. Reticulocyte counts of rabbits in all treatment groups exposed to radiation from either the 1000- or the 250-kvp machine showed extreme variation. The control counts were likewise variable. None of the observed changes are attributable to radiation damage.

4. DISCUSSION

Because of the great variability in counts of the cellular components of the blood of experimental animals under laboratory conditions, a radiation-induced change must be very prominent before any significance can be attached to it. For this reason many of the negative results reported for these studies must be accepted with reservations.

As a rule, the blood counts of man are only slightly less variable than those of experimental animals, and, in considering the hematological picture following accidental exposure to radiation, such inherent variation must always be taken into consideration.

The necessity for depending on statistical analysis to demonstrate changes when the X-ray doses were near the tolerance range is especially significant. Clearly, if a large group of animals must be exposed to a given dose before the significance of a slight effect is demonstrable, no significance can be attached to changes of equally small magnitude in an individual who is accidentally exposed to comparably small doses of radiation. Statistics obviously do not apply in isolated instances. Furthermore, with more prolonged periods of exposure and observation, it might be expected that smaller and smaller doses would prove statistically effective. In this respect the chronic rat experiments might be cited. These studies were excluded from this chapter because of sources of error which tended to obscure trends of small magnitude. The results indicate, however, that 1.0 r/day for 1 year or 0.5 r/day for 2 years produced statistically significant depressions of the lymphocytes. In the dog, 0.5 r/day after a period of 2 years, but not after 1 year, produced a slight, but statistically significant, depression of the lymphocytes. If the experiments had been extended beyond the 2-year period, even smaller doses of X radiation administered repeatedly might have produced significant changes in the hemograms.

The findings in the experiments with rabbits are of importance in that they indicate the variable response which is observed when different species are exposed to like quantities of radiation under comparable conditions. The evaluation of the factor of species variation is uncertain, at best, whenever attempts are made to apply data based on animal studies to human beings.

It is also important in considering these experiments to recognize the fact that changes in a hemogram serve largely to indicate gross damage to the hematopoietic system and that more protracted or subtle changes in this or other systems may not be reflected by any change in the variables selected for routine study.

When the results of these studies are considered in the light of the limitations described above, they indicate clearly that, although routine hematological examination is a valuable adjunct to radiation monitoring, it is not in itself, in the form here described, reliable as a biological monitor in exposures near the tolerance range.

5. SUMMARY AND CONCLUSIONS

Routine hematological examinations have been made repeatedly on dogs, rabbits, rats, and monkeys which have been exposed to daily doses of whole-body X radiation in amounts near the tolerance range, i.e., doses varying from 0.1 to 10.0 r/day. These dosages have been administered continuously over periods extending up to 2 years. Both 250- and 1000-kvp machines were used. During the course of the experiments the rats and monkeys acquired extraneous pathology of such a degree that the observed hematological changes could not reasonably be attributed to the radiation received. For this reason it was necessary to exclude these animals. In general, it is well to exercise caution in interpreting all negative results since the absence of changes which might be observed in routine hematological examinations in no way guarantees that unobserved changes have not occurred.

The significant findings may be summarized briefly as follows:

1. In dogs and rabbits, changes in the hemograms which followed chronic exposure to whole-body X radiation in amounts near the tolerance range were detected only by statistical methods. In view of this finding, it would seem unlikely that routine hematological examination in the form here described would provide a reliable means of detecting minimal radiation damage in any one animal.

2. Lymphocytes appear to be the most sensitive hematological indicator of the damage which results from chronic exposure to X radiation. The least sensitive element in the peripheral blood appears to be the mature erythrocyte.

3. The effectiveness of small daily doses of X radiation in producing hematological changes is dependent, in part, on the duration of such exposure. In some instances, changes were first noted during the second year of daily exposure.

4. The data clearly demonstrate a marked difference in the hematological responses of dogs and rabbits exposed daily to the same doses of radiation. This calls attention to the well-known factor of species variation and indicates its importance whenever attempts are made to apply data based on animal studies to human beings.

5. The reticulocyte depression, relative to that of the lymphocyte, is less pronounced in these experiments than in the case of exposure to single large doses of whole-body X radiation (Chap. 3). Chronic exposure did not elicit the marked reticulocytosis which characterized the recovery response to acute exposure.

6. No consistent difference was observed in the hematological changes which followed exposure to like quantities of X radiation from the 250- and the 1000-kvp machines.

ACKNOWLEDGMENTS

For the statistical evaluations in these studies, the authors wish to acknowledge their appreciation of the extensive assistance of the Statistics Division and, in particular, the cooperation of Dorothy Kuhnert and Helen Baldwin Michaels.

Chapter 12*

PATHOLOGY IN ANIMALS SUBJECTED TO REPEATED DAILY EXPOSURE TO X RAYS

By Roger G. Metcalf and Frank A. Inda

1. FINDINGS IN RATS

1.1 Introduction. The study, in this experiment, of tissue injury in rats exposed to X irradiation administered in doses of 0.1, 0.5, 1.0, and 10 r/day for a period of 2 years has resolved itself into the study of four groups, namely, those animals that died during the course of the 2-year irradiation period, those sacrificed at the termination of irradiation, those sacrificed at the end of the 3-month postirradiation rest period, and those that died during the rest period. Table 12.1 shows the distribution of animals in these four groups according to dose level and voltage. Among those animals that died during the experiment, nine were lost for final tissue study owing to circumstances such as marked autolysis or actual loss of the animal, thus accounting for the apparent discrepancies in the totals listed in the table. Note that there was little difference in the number of deaths with respect to voltage at the various dosages.

1.2 Methods. Animals that died during the experiment were autopsied as soon after death as possible. Ether was used to kill the rats in the sacrificed groups. Tissue was taken from the heart, lung, liver, spleen, pancreas, lymph nodes, stomach, small and large bowels, kidney, adrenal, gonads, thyroid, trachea, skin, and epiphysis of femur and vertebra. Tissues were fixed in Zenker's or Bouin's fluid and embedded in paraffin. Sections were cut 7 μ thick. Harris' hematoxylin and eosin were the stains employed. Bone-marrow smears were taken from the midshaft of a femur and stained by Wright's method.

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*Based on Report UR-88, July 5, 1951. Work done by Roger G. Metcalf, Frank A. Inda, Thomas B. Barnett, and George W. Casarett.

In order to evaluate accurately the various lesions encountered, tables were devised to take into account all variations from normal. As work progressed, it became evident that many lesions listed were of no value (because of their infrequency) in establishing the effect of irradiation upon the animal organs. Therefore the final tables included only the pertinent lesions. Certain terms require explanation; others are self-explanatory. Brief statements concerning the former follow:

Table 12.1 — Grouping of Rats According to Time and Manner of Death*

Dose,	Died during irradiation		Sacrificed at end of irradiation		Sacrificed following rest period		Died during rest period		
r/day	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	1000 kv	250 kv	Total
0.1	32	40	6	4	6	2	5	3	98
0.5	36	35	4	5	1	4	6	5	96
1.0	42	37	3	4	2	2	3	6	99
10.0	25	23							48
Total	135	135	13	13	9	8	14	14	341

* All animals exposed to 10 r/day died during treatment.

Pulmonary infection includes lesions such as pneumonia, interstitial pneumonitis, bronchitis, and bronchiectasis.

Nodular periarteritis is a lesion in rats comparable to the condition in humans known as "periarteritis nodosa." It chiefly affects the mesenteric vessels of the rat. It is characterized histologically by the presence of active fibroblastic proliferation and round-cell infiltration about the vessel. The vessel wall is frequently seen to be undergoing necrosis. Occasionally a thrombus may be seen within the vessel.

Edema of caecal nodes* is a term employed to describe edematous cystic lymph nodes adjacent to the caecum.

Liver granuloma* is used to describe focal chronic granulomatous inflammation in this organ. It differs from parasitic granulomata in that the lesions are smaller and there are no parasites present.

Spleen granuloma* is the same as liver granuloma except for its site.

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* According to Ratcliffe,[1] these lesions are associated with Salmonella infections in the rat.

Leukemia: Lymphatic leukemia was diagnosed when the lymph nodes exhibited an obliteration of normal structure and were infiltrated with nongranular, undifferentiated basophilic cells and lymphocytes. Similar cellular infiltrations were observed in the portal areas of the liver, kidneys, spleen, bone marrow, and, occasionally, other organs. Myeloid leukemia was diagnosed when an infiltration of myeloid cells in varying stages of development was observed in the sinusoids of the liver, spleen, lymph nodes, and, occasionally, other organs. For reasons discussed in Chap. 11, peripheral-blood studies[1] were of little help in establishing the diagnosis of leukemia.

Hemorrhage in the lymph node is used in reference to those nodes showing marked dilation and engorgement of the lymph-node sinuses with blood and blood-filled macrophages. In several instances it was indistinguishable from true hemorrhage. In addition, mononuclear pigment-laden phagocytes were frequently encountered.

Hemorrhage of the adrenal indicates the presence of moderately to markedly dilated spaces containing a fibrinous coagulum, blood cells, or altered blood pigment. Frequently, pigment-laden phagocytes are distributed in the edematous adrenal parenchyma adjacent to the space wall.

Hyperplasia of splenic pulp indicates an increased cellularity of the red pulp. In most instances the term has been used arbitrarily to include the islands of small deeply staining cells and more mature myeloid cells, which represent extramedullary hematopoiesis.

Hypoplasia of splenic follicles refers to a reduction in size of the lymphoid follicles of the spleen. This reduction is apparently caused by a reduced number of lymphocytes.

Hypoplasia of lymph follicles indicates a marked decrease in size or a complete absence of the germinal follicles of the lymph nodes. As in the case of the spleen, the smaller size of the follicles is the result of reduction in the number of lymphocytes.

Atrophy of the testis is a term used to indicate loss of cells in the spermatogenic series, which results in a gradual loss of the more mature forms by maturation until finally the mature spermatozoa are last to disappear. The atrophy of senility and avitaminosis usually progresses in the reverse order. The changes in the testes not showing complete absence of germinal cells (▲+) were apparently of the latter type. These different types of change have been discussed at greater length in Chap. 2.

1.3 Results. (a) Animals That Died During Treatment. Since the animals in this group did not all survive for the same length of time, they did not lend themselves to the facile study and presentation of pathological changes. However, Tables 12.2 to 12.6 show the type and severity of pertinent lesions encountered for each animal, including controls. In Table 12.6, which summarizes the lesions in control animals that died during the treatment period, the line "No. of treatments" indicates the time of death rather than actual treatment. The degree of severity of each lesion is tabulated on the basis of trace to severe. The findings listed are those for rats that died during the treatment period and do not include the sacrificed animals; the latter animals are considered in separate tables. One column shows the frequencies of the various lesions, and another gives the combined frequencies for a group of lesions which are part of the same disease or related diseases. For example, the ulcers of the large bowel together with the granulomata in the spleen and liver and edema of the caecal nodes are considered as lesions peculiar to Salmonella infection of the bowel. The occurrence of multiple lesions in a single disease will explain the apparent discrepancy in the figures in the "Frequency" and "Combined frequency" columns.

These tables show certain interesting possibilities. The presence of pulmonary infection with comparatively equal frequency in rats in the various dosage groups, except the 10 r/day group, is shown. The lower frequency in the 10 r/day group might suggest that the animals in this group were protected from the disease by irradiation. However, it is more likely that the high mortality stemming from other conditions was responsible for this apparent reduction in the incidence of pulmonary infections in the 10 r/day group.

All male animals in the 10 r/day group showed testicular atrophy of a severe grade (Fig. 12.1). This is the only group and only lesion in which consistency of this order has occurred. Atrophy of the testis occurred in other groups in up to 56 per cent of the male animals, but the degree of severity was by no means consistent and was of a type which suggested that some factor other than irradiation was at work in some of the animals (Fig. 12.2). This factor was probably senility or general debility, the ascertaining of which is beyond the scope of this work.

Ulcers of the large bowel occurred more frequently in the 10 r/day group. Although the frequency was less in other groups, there was no striking variation in the degree of severity. Similarly, the other lesions considered to be a part of the Salmonella syndrome followed the pattern of greater frequency in the 10 r/day group.

Hemorrhage in the bone marrow or lymph nodes did not occur among the control animals regardless of the time or cause of death. Its occurrence in these organs in the treated animals that died was erratic (see Tables 12.2 to 12.6), thus making an interpretation difficult if at all practical. However, its absence in controls suggests that the hemorrhage is of significance with respect to the treated animals.

(Text continues on page 290.)

Table 12.2a — Findings in Rats Dying After Exposure to X Rays[a]
(0.1 r per Treatment, 1000 kv)

No. of treatments	158	225	252	266	270	291	294	334	338	346	347	353	403	410	417	442	465	470	488
Animal No.	11	4	22	10	30	3	26	7	1	46	27	16	21	19	31	2	8	17	15
Sex	M	M	F	F	M	M	M	F	M	F	M	M	F	F	F	M	F	M	M
Pulmonary infection	□	△	△		■	△			△	△	△	△	△	△	□	△	△	■	■
Necrosis, liver	b		■																
Chronic nephritis																			
Nodular periarteritis																			
Ulcer																			
Small bowel			b				b			b									b
Large bowel		b	b		■		■												b
Edema, caecal nodes																			
Granuloma																			
Liver	b																		
Spleen						b													
Hemorrhage																			
Bone marrow	b				b														
Lymph node			b										■		b	b			
Adrenal		b	b	b	b		b	b	b	b			□	■					
Hyperplasia, splenic pulp		c	□			b				■	□			□	□			■	
Hypoplasia																			
Splenic follicles		c	□		△	b	△		△	□	■	■	■	□	□	□		□	△
Lymph follicles		c	b	+	d		c	+			+		+		b	b		+	c
Bone marrow	b				b												□		
Atrophy, testis	□				■				□		□	■							△
Reticulum-cell sarcoma		+					+												+
Lymphosarcoma					+														
Lymphoma																+			
Other sarcoma																			
Carcinoma																			
Lung																			
Uterus																			
Skin																			
Ovary																			
Leukemia																			
Lymphatic																			
Myelogenous																			
Fibroadenoma, mammary																			
Other benign tumors																			
Miscellaneous																		g,h	

[a] The following symbols are used in this table to denote the various degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Changes not recorded because of leukemic involvement.

[f] Papilloma, stomach.

[g] Bacterial necrosis, kidney.

[h] Hemorrhage, large bowel.

[i] Ulceration, stomach.

Table 12.2a — (Continued)

No. of treatments	490	510	513	514	526	552	560	577	585	594	613	620	622	Freq.	Combined frequency	
Animal No.	20	28	48	32	47	38	37	41	25	33	5	24	45			
Sex	F	M	F	F	F	M	M	F	M	F	M	M	F			
Pulmonary infection	▲	△	■	△	△		△			■		■	■	25/32		
Necrosis, liver		□	■				■							4/31		
Chronic nephritis		□	△								△	■		4/32		
Nodular periarteritis													■	1/32		
Ulcer																
Small bowel					b									0/27		
Large bowel						■								3/29	6/32	
Edema, caecal nodes						■		△				□		3/32		
Granuloma																
Liver							□							1/31		
Spleen														0/31		
Hemorrhage																
Bone marrow														0/30		
Lymph node				b			b			b				1/26		
Adrenal	▲		■				□			■				6/24		
Hyperplasia, splenic pulp			△	■	■	△	△	▲	c	△	■	△		15/29		
Hypoplasia																
Splenic follicles		■	△	■		■	△		c	■	□	△		21/29		
Lymph follicles	e		+	b	+		b		c	b	+	+	+	10/20		
Bone marrow	e													1/29		
Atrophy, testis		△				■	△		b		■	△		11/16		
Recticulum-cell sarcoma									+					4/32	6/32	7/32
Lymphosarcoma														1/32		
Lymphoma														1/32		
Other sarcoma														0/32		
Carcinoma																
Lung														0/32	0/32	
Uterus														0/15		
Skin														0/32		
Ovary														0/15		
Leukemia																
Lymphatic	+													1/32	1/32	
Myelogenous														0/32		
Fibroadenoma, mammary			+											1/15	2/32	
Other benign tumors								+,f						1/32		
Miscellaneous							i									

[a] The following symbols are used in this table to denote the various degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Changes not recorded because of leukemic involvement.

[f] Papilloma, stomach.

[g] Bacterial necrosis, kidney.

[h] Hemorrhage, large bowel.

[i] Ulceration, stomach.

Table 12.2b — Findings in Rats Dying After Exposure to X Rays[a]
(0.1 r per Treatment, 250 kv)

No. of treatments	205	229	248	248	292	304	328	341	360	369	375	377	398	419	420	424	429	429	444	452	463	472
Animal No.	30	8	33	43	29	49	44	10	22	36	37	45	11	48	4	7	28	46	16	35	40	39
Sex	M	F	F	F	M	F	F	F	M	F	F	F	M	F	M	F	M	F	M	F	F	F
Pulmonary infection	■				△		△	△	■	■	■	△		□	△	■	■		□		□	b
Necrosis, liver				□				b											□	□		b
Chronic nephritis							b										▲					b
Nodular periarteritis																						b
Ulcer																						
Small bowel	b		b			■				b		b	b				b					b
Large bowel	b		b			△				b							b				▲	b
Edema, caecal nodes									□			□	▲								△	b
Granuloma																						
Liver								b		△		□					■					b
Spleen			b					b							b						△	b
Hemorrhage																						
Bone marrow											b			b								b
Lymph node									■											b		b
Adrenal			b	b	b	b	b	b				△				□						b
Hyperplasia, splenic pulp		△	b	c			■	b	■	▲	■	■	■	c	b	△		■	c			b
Hypoplasia																						
Splenic follicles		△	b	c		△	c	b		□	△	■		c	b			□	c			b
Lymph follicles			c	c	+		c	+		+	c	+		c	+	+	+		c	b	+	b
Bone marrow				c			c				b			b					c			b
Atrophy, testis															△							b
Reticulum-cell sarcoma			+	+			+				+			+					+			b
Lymphosarcoma																						b
Lymphoma																						b
Other sarcoma																						b
Carcinoma																						
Lung																						b
Uterus																						b
Skin																						b
Ovary																						b
Leukemia																						
Lymphatic																						b
Myelogenous																						b
Fibroadenoma, mammary																						b
Other benign tumors																						b
Miscellaneous				d																		b

[a] The following symbols are used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Pulmonary edema.

[e] Degeneration, renal cortical tubules.

Table 12.2b — (Continued)

No. of treatments	508	509	522	523	527	545	549	549	552	562	563	566	572	576	576	591	602	611	Freq.	Combined frequency	
Animal No.	24	18	23	1	25	26	2	13	27	17	14	15	50	12	32	31	9	47			
Sex	M	M	M	M	M	M	M	M	M	M	M	M	F	M	F	F	F	F			
Pulmonary infection	■	△		□	■	△			■	□	■		□	■	■	□		□	27/39		
Necrosis, liver					■	■	■				■		■				■		9/38		
Chronic nephritis									▲	▲		■		b					4/37		
Nodular periarteritis																			0/39		
Ulcer																					
Small bowel																			1/33		
Large bowel			▲														△		4/35	14/39	
Edema, caecal nodes	■														■	△	■		8/39		
Granuloma																					
Liver			■											■					5/38		
Spleen			▲										■		b	b	■		4/34		
Hemorrhage																					
Bone marrow																			0/37		
Lymph node																		b	1/37		
Adrenal						□									△		■	□	6/33		
Hyperplasia, splenic pulp	■	△		■	■	■		□	■		■		△		b	b	△		18/30		
Hypoplasia																					
Splenic follicles				□	△	■		□		□					b	b		□	12/30		
Lymph follicles	+		+		+	+	+	+		+		+		+	+	+		b	19/31		
Bone marrow																			0/34		
Atrophy, testis			■	△	■	■				■	■	■		■					9/19		
Recticulum-cell sarcoma																			6/39	7/39	7/39
Lymphosarcoma						+													1/39		
Lymphoma																			0/39		
Other sarcoma																			0/39		
Carcinoma																					
Lung																			0/39	0/39	
Uterus																			0/19		
Skin																			0/39		
Ovary																			0/19		
Leukemia																					
Lymphatic																			0/39	0/39	
Myelogenous																			0/39		
Fibroadenoma, mammary																			0/19	0/39	
Other benign tumors																			0/39		
Miscellaneous																					

[a] The following symbols are used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Pulmonary edema.

[e] Degeneration, renal cortical tubules.

Table 12.3a — Findings in Rats Dying After Exposure to X Rays[a]
(0.5 r per Treatment, 1000 kv)

No. of treatments	168	212	220	229	283	285	301	301	314	314	320	323	329	329	355	363	369	379	385	417
Animal No.	5	24	19	38	3	39	9	30	15	1	2	33	23	10	6	22	31	37	11	34
Sex	M	M	M	F	M	F	F	M	M	M	M	F	M	F	F	M	F	F	M	F
Pulmonary infection	△				■						■	■		△			■	■	△	
Necrosis, liver						b		□												
Chronic nephritis			b			b			△							■				
Nodular periarteritis														△						
Ulcer																				
Small bowel	b		b												b					
Large bowel	b		b								△				b					
Edema, caecal nodes							■	■		□										
Granuloma																				
Liver		□				b					■								■	
Spleen																				
Hemorrhage																				
Bone marrow	b						□												□	■
Lymph node						b	□						b	■			□			
Adrenal	b		b		b	b	b	b	b	b	b	b	b	b					□	
Hyperplasia, splenic pulp	□		△		□			△	□	△	△	■	▲	△	■		□	△	△	
Hypoplasia																				
Splenic follicles	□	■	□		■		□	■		■	■	■	□	■				□	□	
Lymph follicles	+	c	c		c	b				c	+		b	+	d				+	
Bone marrow	b	c			c		□												□	
Atrophy, testis		■						□	□	■	□								■	
Reticulum-cell sarcoma		+	+		+					+										
Lymphosarcoma															+					
Lymphoma																				
Other sarcoma																				
Carcinoma																				
Lung																				
Uterus																				
Skin or mammary																				
Ovary																				
Leukemia																				
Lymphatic																				
Myelogenous																				
Fibroadenoma, mammary																				
Other benign tumors																				
Miscellaneous		g							g					h						

[a] The following symbols are used in this table to indicate varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Fibrosarcoma in abdominal lymph node.

[f] Subcutaneous fibroma.

[g] Moderate pulmonary edema.

[h] Ulceration, stomach.

Table 12.3a — (Continued)

No. of treatments	425	438	443	459	462	471	481	482	494	495	499	519	547	556	561	608	Freq.	Combined frequency	
Animal No.	32	43	42	35	14	49	18	25	12	21	29	27	17	13	20	50			
Sex	F	F	F	F	M	F	M	M	M	M	M	M	M	M	M	F			
Pulmonary infection	△	△	▲	□	□	b	△		□	△		△	□	△	□	□	21/35		
Necrosis, liver		b				b			□	■							3/33		
Chronic nephritis		b	■			b		□	▲			□	▲				7/32		
Nodular periarteritis			□													■	3/36		
Ulcer																			
Small bowel					b	b						□	b				1/30		
Large bowel						b					■	■					3/32	9/36	
Edema, caecal nodes									△		▲	△					6/36		
Granuloma																			
Liver		b				b											3/33		
Spleen						b									b		0/34		
Hemorrhage																			
Bone marrow						b	□										4/34		
Lymph node	b	■				b				■							5/32		
Adrenal		■	△	□		b	b			b				□		□	6/21		
Hyperplasia, splenic pulp		■	■	□	△	b	△	△	■	△	▲	△	△		b		25/34		
Hypoplasia																			
Splenic follicles					■	b	■	△	■		□	△	△	△	b		21/34		
Lymph follicles	b	+			c	b		c	+					+		+	8/25		
Bone marrow					c	b	□										3/31		
Atrophy, testis					■		□	△	■		△	△	■	■	△		15/21		
Reticulum-cell sarcoma					+			+									6/36	7/36	8/36
Lymphosarcoma																	1/36		
Lymphoma																	0/36		
Other sarcoma							+,e										1/36		
Carcinoma																			
Lung																	0/36	0/36	
Uterus																	0/15		
Skin or mammary																	0/36		
Ovary																	0/15		
Leukemia																			
Lymphatic																	0/36	0/36	
Myelogenous																	0/36		
Fibroadenoma, mammary																+	1/15	2/36	
Other benign tumors															+,f		1/36		
Miscellaneous													h						

[a] The following symbols are used in this table to indicate varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Fibrosarcoma in abdominal lymph node.

[f] Subcutaneous fibroma.

[g] Moderate pulmonary edema.

[h] Ulceration, stomach.

Table 12.3b — Findings in Rats Dying After Exposure to X Rays[a]
(0.5 r per Treatment, 250 kv)

No. of treatments	212	219	225	256	268	277	279	299	311	319	354	388	393	399	435	437	443	447	448	449
Animal No.	4	1	23	9	27	30	2	41	35	31	16	5	44	29	28	12	15	17	32	49
Sex	M	M	M	F	M	M	M	M	F	F	M	M	F	M	M	M	M	M	F	F
Pulmonary infection	■	■				△				△	△	■	■	△	△			■	△	△
Necrosis, liver																				
Chronic nephritis														□	△				■	
Nodular periarteritis																				
Ulcer																				
Small bowel		b					b		b	b						b		b		b
Large bowel												b				b		b		b
Edema, caecal nodes																			△	■
Granuloma																				
Liver							■					□		△				■		
Spleen																		b		b
Hemorrhage																				
Bone marrow							■	□	■											
Lymph node							b					△		b	b			b		
Adrenal				b	b	b	b	b	b	b	b							b	■	△
Hyperplasia, splenic pulp		△	c	△			△				□			▲	■	■	□	b	■	b
Hypoplasia																				
Splenic follicles	■	△	c		■		□		△		□		□	■	□			b		b
Lymph follicles		+	c	+		+	b	+			+	+		b	b	+		b		+
Bone marrow			c				■	□	□											
Atrophy, testis	□	△												■	△					
Reticulum-cell sarcoma							+													
Lymphosarcoma																				
Lymphoma																				
Other sarcoma																				
Carcinoma																				
Lung																				
Uterus																				
Skin or mammary																				
Ovary																				
Leukemia																				
Lymphatic			+																	
Myelogenous																				
Fibroadenoma, mammary																				
Other benign tumors						+,f														
Miscellaneous				g																

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[a] The following symbols are used in this table to indicate varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Skin carcinoma.

[e] Mammary carcinoma.

[f] Subcutaneous fibroma.

[g] Large abscess in abdominal node.

Table 12.3b—(Continued)

No. of treatments	477	485	491	503	511	521	535	554	561	572	581	584	588	592	619	Freq.	Combined frequency	
Animal No.	18	36	26	50	45	20	11	46	21	33	47	6	37	43	34			
Sex	M	F	M	F	F	M	M	F	F	F	F	F	F	F	F			
Pulmonary infection	△		□	■	□	△	□	△	■	b	■	△	□		△	24/34		
Necrosis, liver								■		b						1/34		
Chronic nephritis						■				▲				■	△	7/35		
Nodular periarteritis														■		1/35		
Ulcer																		
Small bowel										b				b		0/26		
Large bowel				△	▲		▲	△		b			▲			5/30	13/35	
Edema, caecal nodes					■		▲							□		5/35		
Granuloma																		
Liver		△		□	■		△	□		b				□		10/34		
Spleen					■		■			b						2/32		
Hemorrhage																		
Bone marrow															△	4/35		
Lymph node									□							2/31		
Adrenal		△						□	b	■	△					6/25		
Hyperplasia, splenic pulp	□	▲	c		△	■	△	▲	□	b	△	△	△	□	□	21/30		
Hypoplasia																		
Splenic follicles	■		c		△		□	▲	■	b		□	■	□	■	18/30		
Lymph follicles		+	c	+		+	+	+		+	+	+	+	+	+	19/29		
Bone marrow			c		□											4/33		
Atrophy, testis	△															5/18		
Reticulum-cell sarcoma																1/35	1/35	6/35
Lymphosarcoma																0/35		
Lymphoma																0/35		
Other sarcoma																0/35		
Carcinoma																		
Lung																0/35	3/35	
Uterus				+												1/17		
Skin or mammary													+,d	+,e		2/35		
Ovary																0/17		
Leukemia																		
Lymphatic			+													2/35	2/35	
Myelogenous																0/35		
Fibroadenoma, mammary																0/17	2/35	
Other benign tumors														+, f		2/35		
Miscellaneous																		

[a] The following symbols are used in this table to indicate varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Skin carcinoma.

[e] Mammary carcinoma.

[f] Subcutaneous fibroma.

[g] Large abscess in abdominal node.

Table 12.4a — Findings in Rats Dying After Exposure to X Rays[a]
(1 r per Treatment, 1000 kv)

No. of treatments	192	256	269	274	281	287	306	317	325	343	351	381	388	410	411	417	418	420	426	442	446	459	459
Animal No.	9	10	3	21	16	5	6	2	37	38	48	33	31	44	32	4	42	24	14	11	12	39	43
Sex	M	F	M	M	M	M	F	M	F	F	F	F	F	F	F	M	F	M	M	M	M	F	F
Pulmonary infection	□	■		△			□	△	△	■				△	△	□	■	□	▲	b	△		
Necrosis, liver													□						■				
Chronic nephritis																		▲			△		△
Nodular periarteritis																					△		
Ulcer																							
Small bowel	b								b													b	
Large bowel																▲		△					
Edema, caecal nodes						△															■		
Granuloma																							
Liver						□		■								□				□			■
Spleen								△															
Hemorrhage																							
Bone marrow																							
Lymph node					■					△	■				□								
Adrenal	b	b	b	b	b	b	b	b		b		■		■				b	b			■	■
Hyperplasia, splenic pulp		▲	■	■		▲	■			c	■	▲	△	■	■	▲			■	b		△	■
Hypoplasia																							
Splenic follicles	△	□	△	□		▲	■		□		■	■	□		■	■		■	■	b			■
Lymph follicles	c	c	c	+		+		+	+	c		+	+				+	+		+			
Bone marrow	c		□							c													
Atrophy, testis			△																■				
Reticulum-cell sarcoma	+	+	+							+											+		
Lymphosarcoma																							
Lymphoma																							
Other sarcoma						+,d							+,e										
Carcinoma																							
Lung																							
Uterus																							
Skin or mammary																							
Ovary																							
Leukemia																							
Lymphatic																							
Myelogenous																							
Fibroadenoma, mammary																							+
Other benign tumors																							
Miscellaneous																						i	

[a] The following symbols are used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Osteogenic sarcoma.

[e] Subcutaneous sarcoma.

[f] Sarcoma in lung, kidney, bowel, and ovary.

[g] Mammary carcinoma.

[h] Subcutaneous fibroma.

[i] Moderate pulmonary edema.

Table 12.4a — (Continued)

No. of treatments	464	469	470	471	475	489	501	501	513	536	549	576	577	580	594	600	601	618	621	Freq.	Combined frequency
Animal No.	50	46	30	23	15	7	47	8	36	40	49	45	25	29	1	26	28	34	20		
Sex	F	F	M	M	M	F	F	F	F	F	F	F	M	M	M	M	M	F	M		
Pulmonary infection	△	■	△	■	△		△	△			b	■	□	□	△	□		△	■	28/40	
Necrosis, liver		□												△	□		■			6/42	
Chronic nephritis				□			△					□			▲			△	△	9/42	
Nodular periarteritis																				1/42	
Ulcer																					
Small bowel			b																	0/38	
Large bowel			■					■			b				△		△			5/40	12/42
Edema, caecal nodes								■												3/42	
Granuloma																					
Liver					■	△		□									■			9/42	
Spleen					■	▲														3/42	
Hemorrhage																					
Bone marrow				■								□								2/42	
Lymph node					■			□				□								7/42	
Adrenal		■					b	■	■		b			b				△		8/28	
Hyperplasia, splenic pulp	▲	△	□	□	▲	△	▲	△	△	▲	■	■		△	■	□	□		□	31/40	
Hypoplasia																					
Splenic follicles	□	▲	□	■	■		■	■		△	■	■	△		■	■		■	□	30/41	
Lymph follicles	+	c				+	b		■	+	+	+		+	+		+	+	+	19/35	
Bone marrow				□																2/40	
Atrophy, testis			□	■	■								□		■	■			■	9/20	
Reticulum-cell sarcoma		+													+					7/42	7/42 12/42
Lymphosarcoma																				0/42	
Lymphoma																				0/42	
Other sarcoma	+,e									+,f										4/42	
Carcinoma																					
Lung																				0/42	1/42
Uterus																				0/22	
Skin or mammary							+,g													1/42	
Ovary																				0/22	
Leukemia																					
Lymphatic																				0/42	0/42
Myelogenous																				0/42	
Fibroadenoma, mammary						+														2/22	3/42
Other benign tumors													+,h							1/42	
Miscellaneous																	i				

[a] The following symbols are used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Osteogenic sarcoma.

[e] Subcutaneous sarcoma.

[f] Sarcoma in lung, kidney, bowel, and ovary.

[g] Mammary carcinoma.

[h] Subcutaneous fibroma.

[i] Moderate pulmonary edema.

Table 12.4b — Findings in Rats Dying After Exposure to X Rays[a]
(1 r per Treatment, 250 kv)

No. of treatments	44	216	283	294	304	323	330	354	363	369	370	374	381	393	398	405	411	417	420	422	429
Animal No.	16	44	9	5	27	12	4	33	50	7	6	3	23	34	41	18	22	1	24	15	21
Sex	M	F	F	M	M	M	M	F	F	F	F	M	M	F	F	M	M	M	M	M	M
Pulmonary infection	△			△				△					△			△	□	□	△		□
Necrosis, liver						■				b		■					□				△
Chronic nephritis				□															■	□	
Nodular periarteritis																					
Ulcer																					
Small bowel		b									b				b			b			
Large bowel									▲		b				△						
Edema, caecal nodes									△												
Granuloma																					
Liver									□									■			
Spleen																					
Hemorrhage																					
Bone marrow	b							□								■					
Lymph node																			□		■
Adrenal			b		b	b	b		■	b		b	□	□	b				b		b
Hyperplasia, splenic pulp		c	d			■	△		△	■	△	c	■	△	□		■		■		
Hypoplasia																					
Splenic follicles	■	c	d		□	■		■			■	c	■				■	□	□	■	△
Lymph follicles	+	c			b	d	d	d		+	+	c	b		b		+	+	+		+
Bone marrow	b	c	d			d	d	□				c									
Atrophy, testis						□,e										■		b		△	
Reticulum-cell sarcoma			+			+	+	+													
Lymphosarcoma																					
Lymphoma																					
Other sarcoma																					
Carcinoma																					
Lung																					
Uterus																					
Skin or mammary																				+,f	
Ovary																					
Leukemia																					
Lymphatic												+									
Myelogenous		+																			
Fibroadenoma, mammary																					
Other benign tumors																					
Miscellaneous											i									j	

[a] The following symbols have been used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement by reticulum-cell sarcoma.

[e] Intertubular hemorrhage, testis.

[f] Skin carcinoma.

[g] Papillary cystadenoma, ovary.

[h] Subcutaneous fibroma.

[i] Large abscess, oviduct.

[j] Ulceration, stomach.

Table 12.4b — (Continued)

No. of treatments	432	442	443	471	484	497	498	514	544	557	578	581	597	599	601	608	Freq.	Combined frequency
Animal No.	47	39	20	42	40	32	36	29	26	28	48	8	31	37	11	13		
Sex	F	F	M	F	F	F	F	M	M	M	F	M	F	F	M	M		
Pulmonary infection	△		△		△	△		△			■	■		■	■	□	19/37	
Necrosis, liver						△								□			6/36	
Chronic nephritis			□			■	△	△		▲	△						9/37	
Nodular periarteritis						▲	■			□							3/37	
Ulcer																		
Small bowel		b									■					b	1/31	
Large bowel		▲	■						△		■					▲	7/36	12/37
Edema, caecal nodes			■	■						△	■	■					6/37	
Granuloma																		
Liver		△						■			△						5/37	
Spleen		b															0/36	
Hemorrhage																		
Bone marrow			■														3/36	
Lymph node	■																3/37	
Adrenal		b				△			b	△							5/26	
Hyperplasia, splenic pulp	□	b	□	▲	c		□	■	■	■		△		△		□	20/32	
Hypoplasia																		
Splenic follicles	□	b	■	■	c		■	■	■			■	■		□	□	21/32	
Lymph follicles	+	b		+	c	+		b	+	+		+	+		+	+	16/26	
Bone marrow			□		c												2/30	
Atrophy, testis			△,e						▲			b					5/18	
Reticulum-cell sarcoma								+									5/37	5/37 10/37
Lymphosarcoma																	0/37	
Lymphoma																	0/37	
Other sarcoma																	0/37	
Carcinoma																		
Lung																	0/37	2/37
Uterus							+										1/17	
Skin or mammary																	1/37	
Ovary																	0/37	
Leukemia																		
Lymphatic																	1/37	3/37
Myelogenous					+												2/37	
Fibroadenoma, mammary							+				+			+			3/17	5/37
Other benign tumors				+, g									+, h				2/37	
Miscellaneous																		

[a] The following symbols have been used in this table to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement by reticulum-cell sarcoma.

[e] Intertubular hemorrhage, testis.

[f] Skin carcinoma.

[g] Papillary cystadenoma, ovary.

[h] Subcutaneous fibroma.

[i] Large abscess, oviduct.

[j] Ulceration, stomach.

Table 12.5a — Findings in Rats Dying After Exposure to X Rays[a]
(10 r per Treatment, 1000 kv)

No. of treatments	191	277	281	298	314	323	328	334	334	341	343	357	369	375	381	395
Animal No.	18	10	14	2	6	3	16	24	7	15	1	4	12	22	25	20
Sex	F	M	M	M	M	F	F	F	M	M	M	F	M	F	F	F
Pulmonary infection	□	□			□	△		△			△	▲				
Necrosis, liver			□													
Chronic nephritis								△								
Nodular periarteritis																
Ulcer																
Small bowel				b										b		
Large bowel				b			□	■	■							□
Edema, caecal nodes									■						■	■
Granuloma																
Liver	■							■			□				■	
Spleen	b												b		■	
Hemorrhage																
Bone marrow			b	b								□				
Lymph node			■			b	b									
Adrenal	b	b	b	b	b	b	b	b	b	b		b		□	■	△
Hyperplasia, splenic pulp	b			c	c	■	c	△	■			△	b	△	△	△
Hypoplasia																
Splenic follicles	b	△	△	c	c	■	c	■	■	□	■	□	b	△		□
Lymph follicles		d	+		c	b	b	+		+	+	+	+	+	+	+
Bone marrow		d	b	b	c							□			c	
Atrophy, testis		▲	▲	e	▲				▲	▲	▲		▲			
Reticulum-cell sarcoma		+														
Lymphosarcoma																
Lymphoma																
Other sarcoma																+,f
Carcinoma																
Lung																
Uterus																
Skin																
Ovary																
Leukemia																
Lymphatic				+	+											
Myelogenous							+								+	
Fibroadenoma, mammary												+				+
Other benign tumors																
Miscellaneous			h													

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[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement of reticulum-cell sarcoma.

[e] No section of testis available. Epididymis contained no sperm.

[f] Fibrosarcoma in abdominal cavity involving kidney.

[g] Adenoma, liver.

[h] Moderately severe pulmonary edema.

[i] Absence of ovarian follicles (two sections examined).

Table 12.5a — (Continued)

No. of treatments	395	405	405	428	430	462	465	525	531	Frequency	Combined frequency
Animal No.	5	17	8	11	23	21	13	9	19		
Sex	F	F	M	M	F	F	M	M	F		
Pulmonary infection		■								8/25	
Necrosis, liver							△			2/25	
Chronic nephritis				▲		■		b	△	4/24	
Nodular periarteritis				□						1/25	
Ulcer											
Small bowel								b		0/22	
Large bowel	■							b		5/23	
Edema, caecal nodes		■								4/25	9/25
Granuloma											
Liver										4/25	
Spleen								b		1/22	
Hemorrhage											
Bone marrow	□				□				■	4/23	
Lymph node				■		□		b		3/22	
Adrenal	■	b		■	■	△		b	△	8/12	
Hyperplasia, splenic pulp		□	c	▲		△	c	b	△	11/17	
Hypoplasia											
Splenic follicles	■	■	c	△	△	△	c	b	△	16/17	
Lymph follicles		+	c	+		+	c	b		12/18	
Bone marrow	□		c		■	□	c		△	5/18	
Atrophy, testis			▲	▲			▲	▲		11/11	
Reticulum-cell sarcoma										1/25	
Lymphosarcoma										0/25	1/25
Lymphoma										0/25	
Other sarcoma										1/25	
Carcinoma											
Lung										0/25	
Uterus										0/13	9/25 8/25
Skin										0/25	
Ovary										0/13	
Leukemia											
Lymphatic			+				+			4/25	6/25
Myelogenous										2/25	
Fibroadenoma, mammary		+								3/13	4/25
Other benign tumors				+, g						1/25	
Miscellaneous					i	h			i		

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement of reticulum-cell sarcoma.

[e] No section of testis available. Epididymis contained no sperm.

[f] Fibrosarcoma in abdominal cavity involving kidney.

[g] Adenoma, liver.

[h] Moderately severe pulmonary edema.

[i] Absence of ovarian follicles (two sections examined).

Table 12.5b — Findings in Rats Dying After Exposure to X Rays[a]
(10 r per Treatment, 250 kv)

No. of treatments	202	262	287	307	328	333	344	373	375	383	386	387	387	387	407
Animal No.	4	19	14	5	13	3	6	23	25	21	9	12	15	22	2
Sex	M	F	M	M	M	F	M	F	F	F	M	M	M	F	F
Pulmonary infection					b		■								
Necrosis, liver		△			□										
Chronic nephritis					b								▲	□	
Nodular periarteritis															
Ulcer															
Small bowel				■				□							b
Large bowel	■		■	△			■	△	□		■	■			
Edema, caecal nodes			△				■								
Granuloma															
Liver	□		■	■			■	□			□			■	□
Spleen									□		□	□			
Hemorrhage															
Bone marrow		□	□										□		
Lymph node				b						b					
Adrenal		b	b	b	b	△	■	△	△	□	■	△		■	■
Hyperplasia, splenic pulp			■	□	△		■		△	■	■	△	△	□	△
Hypoplasia															
Splenic follicles	△	▲	■	△	□		■	■	■		■	■	△	■	△
Lymph follicles	+	+		b	+	+		+	+	b	+	+	+		c
Bone marrow		■	□										■		
Atrophy, testis	▲		▲	▲	▲		▲				▲	▲	▲		
Reticulum-cell sarcoma															+
Lymphosarcoma															
Lymphoma															
Other sarcoma															
Carcinoma															
Lung															
Uterus															
Skin															
Ovary															
Leukemia															
Lymphatic															
Myelogenous															
Fibroadenoma, mammary								+						+	+
Other benign tumors													+,d		
Miscellaneous													e		

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[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement of reticulum-cell sarcoma.

[d] Myxoma, testis.

[e] Moderately severe ulceration of trachea.

[f] Moderately severe pulmonary edema.

Table 12.5b — (Continued)

No. of treatments	430	443	447	449	453	460	477	541	Frequency	Combined frequency	
Animal No.	1	11	16	17	24	18	20	7			
Sex	F	M	F	F	F	F	F	M			
Pulmonary infection	■					■			3/22		
Necrosis, liver								△	3/23		
Chronic nephritis									2/22		
Nodular periarteritis									0/23		
Ulcer											
Small bowel				b					2/21		
Large bowel	△	▲	■			△	■		13/23	17/23	
Edema, caecal nodes	■	▲				■			5/23		
Granuloma											
Liver			■	□	□	■	■		13/23		
Spleen		■		□		△			6/23		
Hemorrhage											
Bone marrow									3/23		
Lymph node									0/21		
Adrenal	■	□		■	□	△	■	□	16/19		
Hyperplasia, splenic pulp	△	△	□	■	■	▲	■	■	19/23		
Hypoplasia											
Splenic follicles	□	△	□	■	△	▲	■	■	21/23		
Lymph follicles		+		+	+	+		+	14/20		
Bone marrow									3/23		
Atrophy, testis		▲						▲	10/10		
Reticulum-cell sarcoma									1/23	1/23	1/23
Lymphosarcoma									0/23		
Lymphoma									0/23		
Other sarcoma									0/23		
Carcinoma											
Lung									0/23	0/23	
Uterus									0/13		
Skin									0/23		
Ovary									0/13		
Leukemia											
Lymphatic									0/23	0/23	
Myelogenous									0/23		
Fibroadenoma, mammary			+		+		+		6/13	7/23	
Other benign tumors									1/23		
Miscellaneous					f						

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement of reticulum-cell sarcoma.

[d] Myxoma, testis.

[e] Moderately severe ulceration of trachea.

[f] Moderately severe pulmonary edema.

Table 12.6 — Findings in Control Rats Dying During Treatment Period[a]

No. of treatments	214	230	238	240	256	256	298	302	351	369	387	390	408	423	451	455	459	463	481	503
Animal No.	21	23	29	27	15	45	7	28	22	30	1	3	35	19	18	42	17	13	43	4
Sex	M	M	M	M	M	F	F	M	M	M	M	M	F	M	M	F	M	M	F	M
Pulmonary infection	□	□	□	■		■	△	△	△	■	△	▲		■	△		▲	□	△	■
Necrosis, liver																				
Chronic nephritis	b								b		△		b							▲
Nodular periarteritis																				
Ulcer																				
Small bowel	b		■		b															
Large bowel	b		▲		b				b				b							
Edema, caecal nodes	■		■																□	
Granuloma																				
Liver			■				■		■											
Spleen		b	□											■	b					
Hemorrhage																				
Bone marrow													b							
Lymph node		b		b											□					
Adrenal		b	b	b			b	b	b				b		■					
Hyperplasia, splenic pulp		b	■	□	c	■	□		■		□		c		b	△	■	■	■	
Hypoplasia																				
Splenic follicles		b	■		c	□	□		■			△	c		b		□			□
Lymph follicles	+	b	+	b	c		+		+			+	c		+	+	+			+
Bone marrow													b							
Atrophy, testis			□						■	b		△			△		■			
Reticulum-cell sarcoma					+								+							+
Lymphosarcoma																				
Lymphoma																				
Other sarcoma																				
Carcinoma																				
Lung																+				
Uterus																				
Skin																				
Ovary																+				
Leukemia																				
Lymphatic																				
Myelogenous																				
Fibroadenoma, mammary																				
Other benign tumors																				
Miscellaneous					g								h		i					

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by tumor.

[e] Osteogenic sarcoma.

[f] Fibroma, skin; hemangioma, mediastinum.

[g] Moderate pulmonary edema.

[h] Moderate pulmonary hemorrhage.

[i] Moderate hemorrhage, gastric mucosa.

Table 12.6 — (Continued)

No. of treatments	509	523	523	531	543	565	567	605	610	610	610	613	614	619	620		
Animal No.	6	46	49	32	36	50	26	37	11	12	16	31	41	47	2	Freq.	Combined frequency
Sex	F	F	F	F	F	F	M	F	M	F	M	F	F	F	M		
Pulmonary infection	■		△		□	■	■		△			■	△	△		26/35	
Necrosis, liver	b									□						1/34	
Chronic nephritis			□		■	■						□				6/32	
Nodular periarteritis										■						1/35	
Ulcer																	
Small bowel																1/33	
Large bowel										△						2/31	9/35
Edema, caecal nodes																2/35	
Granuloma																	
Liver	b		■													4/35	
Spleen											■					3/33	
Hemorrhage																	
Bone marrow																0/34	
Lymph node			b	b	b		b									1/29	
Adrenal		b	△	□				□		□			■	■		7/26	
Hyperplasia, splenic pulp	■		■	■	△					■	■			△		17/30	
Hypoplasia																	
splenic pulp	□	□	■		□				□		■		□	□		15/30	
Lymph follicles	+		b	b	b		b	+	+		+	+	+	+	d	16/26	
Bone marrow															d	0/33	
Atrophy, testis							b				■					6/17	
Reticulum-cell sarcoma																3/35	3/35 6/35
Lymphosarcoma																0/35	
Lymphoma																0/35	
Other sarcoma															+, e	1/35	
Carcinoma																	
Lung																1/35	2/35
Uterus					+											1/16	
Skin																0/35	
Ovary																1/16	
Leukemia																	
Lymphatic																0/35	0/35
Myelogenous																0/35	
Fibroadenoma, mammary										+						1/16	2/35
Other benign tumors							+,f									1/35	
Miscellaneous																	

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by tumor.

[e] Osteogenic sarcoma.

[f] Fibroma, skin; hemangioma, mediastinum.

[g] Moderate pulmonary edema.

[h] Moderate pulmonary hemorrhage.

[i] Moderate hemorrhage, gastric mucosa.

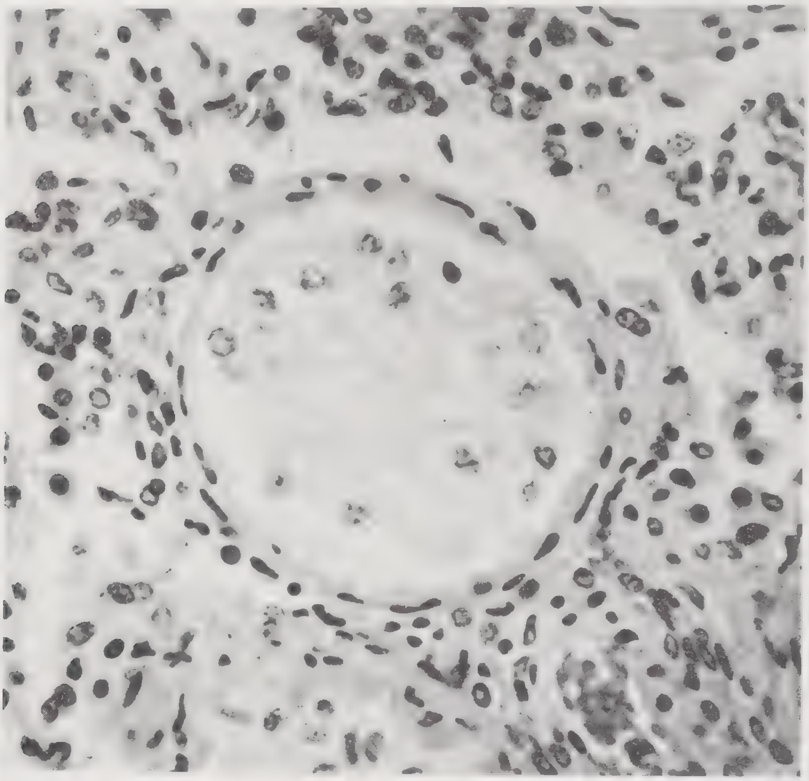

Fig. 12.1—Section of testis from rat A12 which died after 369 treatments at 10 r per treatment. The seminiferous tubules are markedly atrophic and are completely devoid of germinal epithelial cells and spermatozoa. 440×

In approximately 25 to 33 per cent of the animals that died during the experiment, no adrenal sections were present for study. Of the sections taken from all controls that died, approximately 25 per cent showed adrenal hemorrhage (Fig. 12.3). This lesion occurred three times as frequently in the 10 r/day group when compared with the same lesion in other groups, including the controls.

Hypoplasia of the splenic follicles was seen in increasing numbers of animals as the irradiation dose increased until, in the 10 r/day group, 93 per cent of the rats exhibited this change (Fig. 12.4). Fifty per cent of the control animals of this group showed the lesion. However, most examples were mild.

It is doubtful that the hyperplasia in the splenic pulp is significant since it was found so frequently in control animals and the percentage increase in the treated animals is not sufficient to add weight to the possibility that it can be increased by chronic irradiation.

There was no evidence of bone-marrow hypoplasia in the control animals that died during the treatment period. In the treated animals

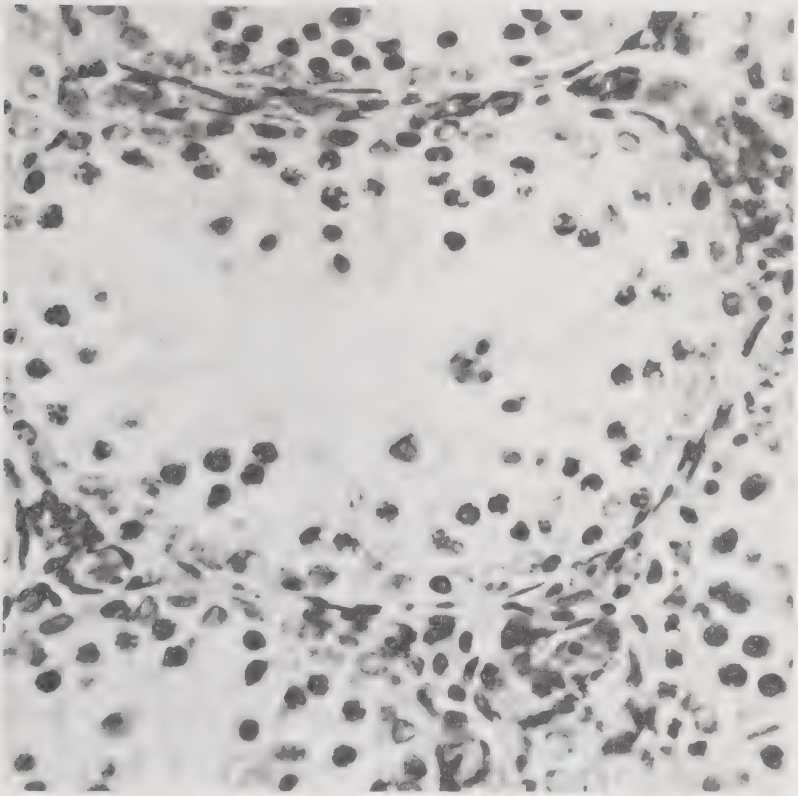

Fig. 12.2 — Section of testis from control rat 3 which died during the experiment. This section illustrates the type of nonspecific testicular atrophy which was observed in control rats and in those exposed at the lower dosage levels. There is a marked reduction in germinal cells which affects the more mature elements to a greater extent than the spermatogonia and primary spermatocytes. One multinucleated cell is shown. 440×

the lesion was found most frequently in the 10 r/day group, reaching 20 per cent at that dose. However, it was of mild to moderate degree.

No definite pattern was apparent in the frequency of malignant tumors exclusive of leukemia. No instance of leukemia was encountered in the control animals. Among the treated animals the number of cases of leukemia increased with the dosage. Approximately 1 per cent of the 0.1 r/day group, 2 per cent of the 0.5 r/day group, 3 per cent of the 1.0 r/day group, and 12 per cent of the 10 r/day group showed this disease. Animals showing features of lymphatic leukemia (Fig. 12.5) were more numerous than those showing features of myeloid leukemia (Fig. 12.6) by a ratio of 2 to 1. The cases of leukemia in any given dose group developed in about 14 to 19 months.

Among the benign tumors encountered, fibroadenoma of the mammary gland (Fig. 12.7) was the most frequent. One of the sixteen (or

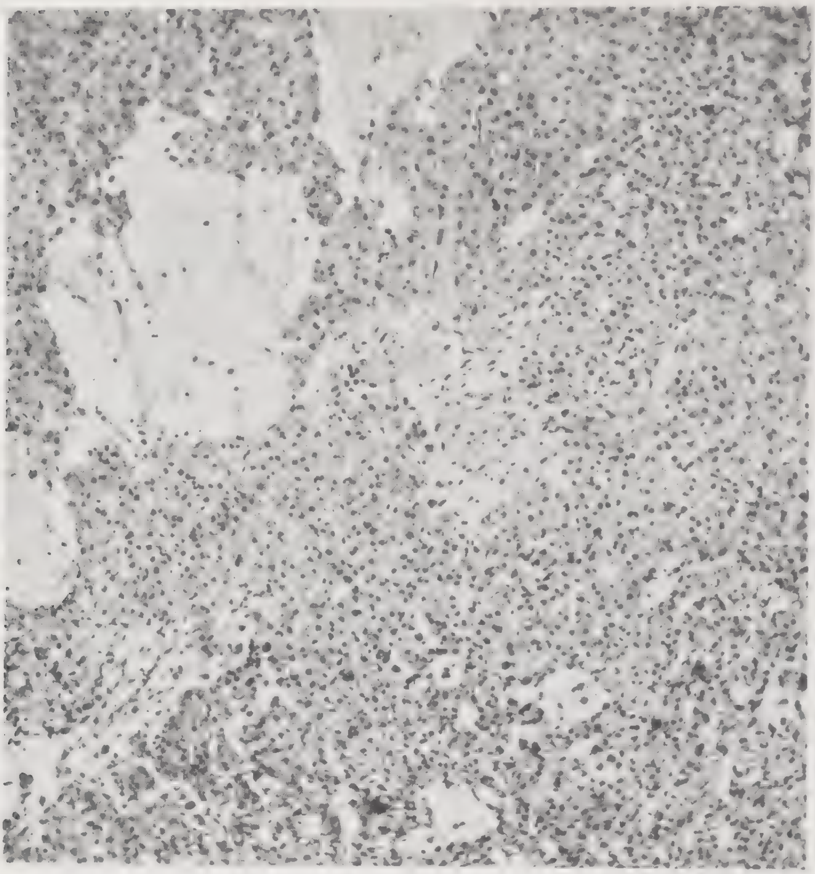

Fig. 12.3—Section of adrenal from rat B18 which died after 460 treatments at 10 r per treatment. Note the dilated spaces containing fibrinous coagulum and blood. 100×

6 per cent) female control animals showed this lesion. In the treated groups the number of fibroadenomata of the mammary gland increased with dosage as follows: 3 per cent in the 0.1 r/day group, 13 per cent in the 0.5 r/day group, 23.4 per cent in the 1.0 r/day group, and 34.5 per cent in the 10 r/day group.

Various types of other benign tumors occurred in all groups. These tumors are identified in the footnotes of the tables. In none of the five groups of animals was their frequency sufficiently great to be significant.

(b) Sacrificed Animals. The small number of animals sacrificed after 2 years of X radiation does not permit fruitful comparison of the various dosage groups. Tables 12.7a and b show the severity of the pertinent lesions and their frequencies.

Those cases of testis atrophy recorded are such that it is uncertain whether irradiation has any relation to the lesion. None of the testis

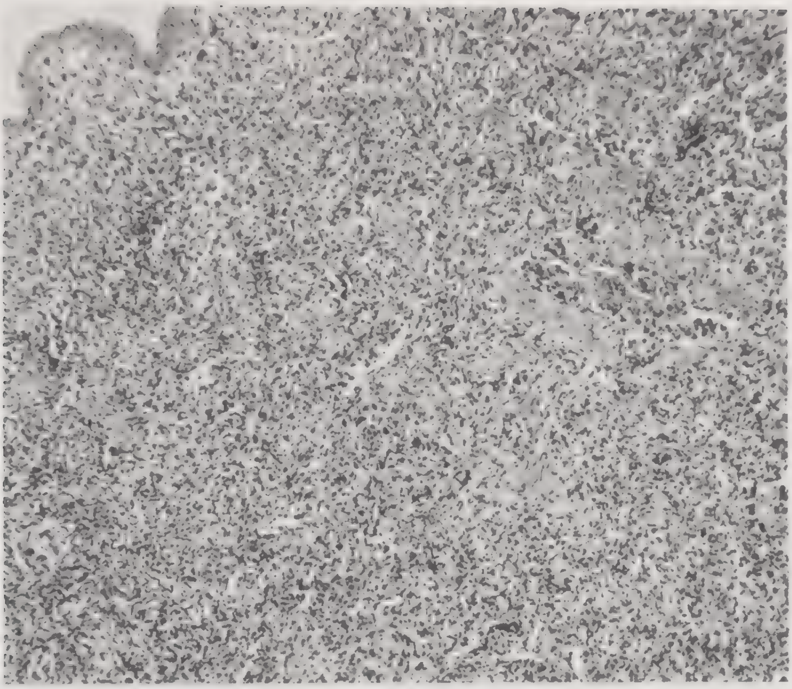

Fig. 12.4—Section of spleen from rat A10 which died after 277 treatments at 10 r per treatment. There is a marked decrease in the cellularity of both the red and white pulp. 100×

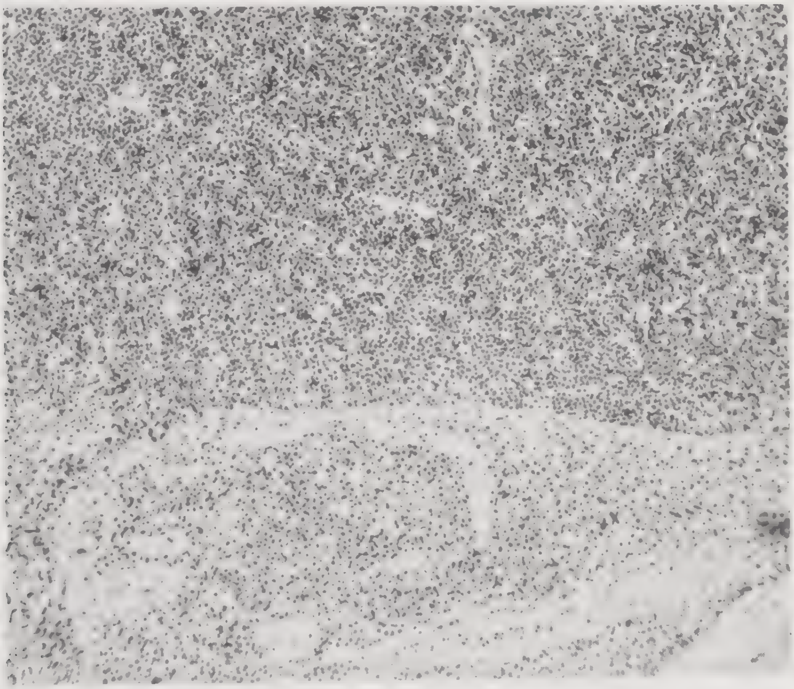

Fig. 12.5—Lymph node from rat A8 which died of lymphatic leukemia after 405 treatments at 10 r per treatment. Note the complete loss of the normal architecture of the node as a result of the massive accumulation of leukemic cells. 100×

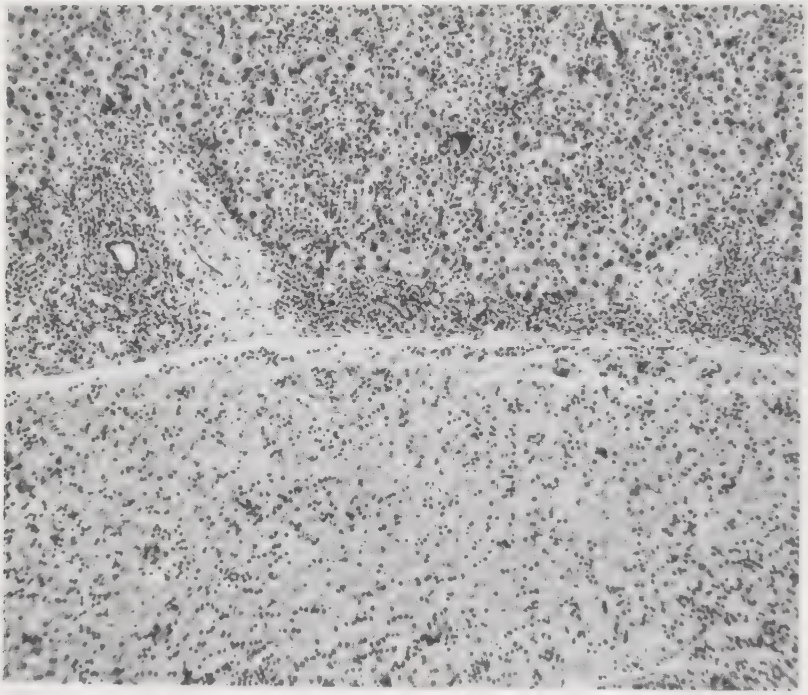

Fig. 12.6—Section of liver from rat B40 which died of myeloid leukemia after 484 treatments at 1 r per treatment. There is marked dilation of the liver sinuses and infiltration of leukemic cells. 100×

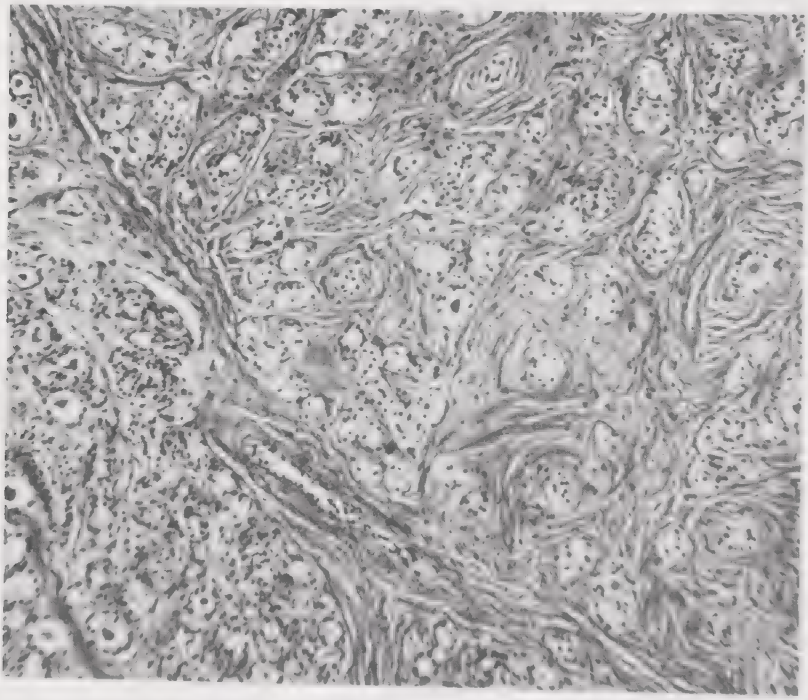

Fig. 12.7—Section of fibroadenoma of the mammary gland from rat A17 which died after 405 treatments at 10 r per treatment. 100×

Table 12.7a — Findings in Rats Sacrificed Immediately After 622 X-ray Exposures[a]

Dose per treatment	Control						0.1 r, 1000 kv							0.1 r, 250 kv				
Animal No.	5	10	14	34	44	Freq.	6	9	13	14	36	49	Freq.	3	21	38	42	Freq.
Sex	M	F	M	F	F		F	F	M	M	M	F		M	M	F	F	
Pulmonary infection					△	1/5			■	■	■		3/6	△	□	■	■	4/4
Necrosis, liver																		
Chronic nephritis										△			1/6	△	△			2/4
Nodular periarteritis											□		1/6	□				1/4
Ulcer																		
Small bowel																		
Large bowel							■						1/6					
Edema, caecal nodes				□		1/5	■						1/6					
Granuloma																		
Liver																		
Spleen																		
Hemorrhage																		
Bone marrow																		
Lymph node									b				0/5	■				1/4
Adrenal							■	■					2/6			■		1/4
Hyperplasia, splenic pulp	■	■	△	△	■	5/5	■	△	■	□	■	△	6/6	■	□	■	■	4/4
Hypoplasia																		
Splenic follicles																		
Lymph follicles		+	+	+	+	4/5	+	+	b	+	+	+	5/5	+	+	+	+	4/4
Bone marrow																		
Atrophy, testis																		
Reticulum-cell sarcoma										+			1/6					
Lymphosarcoma																		
Lymphoma																		
Other sarcoma																		
Carcinoma																		
Lung																		
Uterus																		
Skin																		
Ovary																		
Leukemia																		
Lymphatic																		
Myelogenous																		
Fibroadenoma, mammary												+	1/3					
Other benign tumors																		

[a] The following symbols are used to denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Small adenoma near hilus of kidney (adrenal).

Table 12.7a — (Continued)

Dose per treatment	0.5 r, 1000 kv					0.5 r, 250 kv						1 r, 1000 kv				1 r, 250 kv				
Animal No.	7	16	26	46	Freq.	7	8	13	25	48	Freq.	13	19	41	Freq.	19	30	38	49	Freq.
Sex	F	M	M	F		F	F	M	M	F		M	M	F		M	M	F	F	
Pulmonary infection				□	1/4	△	■			□	3/5			□	1/3	△	□		□	3/4
Necrosis, liver																				
Chronic nephritis													△		1/3					
Nodular periarteritis													□		1/3			□		1/4
Ulcer																				
Small bowel																				
Large bowel																				
Edema, caecal nodes			□		1/4			△			1/5									
Granuloma																				
Liver																	□			1/4
Spleen																				
Hemorrhage																				
Bone marrow			b		0/3							b			0/2			b	b	0/2
Lymph node																				
Adrenal				b	0/3	■	■		b		2/4							b	△	1/3
Hyperplasia, splenic pulp	△		■	△	3/4	■	■	□	□	△	5/5	□	■	■	3/3	△	△	□	△	4/4
Hypoplasia																				
Splenic follicles																				
Lymph follicles	+	+	+	+	4/4	+	+	+	+	+	5/5	+	+	+	2/3		+	+		2/4
Bone marrow			b		0/3							b			0/2			b	b	0/2
Atrophy, testis													■		1/2					
Reticulum-cell sarcoma																				
Lymphosarcoma																				
Lymphoma																				
Other sarcoma																				
Carcinoma																				
Lung																				
Uterus																				
Skin																				
Ovary																				
Leukemia																				
Lymphatic																				
Myelogenous																				
Fibroadenoma, mammary										+	1/3							+	+	2/2
Other benign tumors							+,[c]				1/5									

[a] The following symbols are used to denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Small adenoma near hilus of kidney (adrenal).

Table 12.7b — Findings in Rats Sacrificed 3 Months After 622 X-ray Exposures[a]

Dose per treatment	Control				0.1 r, 1000 kv							0.1 r, 250 kv		
Animal No.	33	38	48	Freq.	12	23	35	40	43	50	Freq.	6	20	Freq.
Sex	F	F	F		M	M	M	M	F	F		F	M	
Pulmonary infection		■		1/3	■			■		■	3/6	■	■	2/2
Necrosis, liver														
Chronic nephritis						■	■	△	■	△	5/6	□		1/2
Nodular periarteritis														
Ulcer														
Small bowel														
Large bowel												■		1/2
Edema, caecal nodes						□	□	□			3/6			
Granuloma														
Liver												■		1/2
Spleen														
Hemorrhage														
Bone marrow		b		0/2								b		0/1
Lymph node												b		0/1
Adrenal			□	1/3				□	□		2/6			
Hyperplasia, splenic pulp	■	□	■	3/3	□	□	■	■	△	■	6/6	△		1/2
Hypoplasia														
Splenic follicles														
Lymph follicles	+			1/3						+	1/6	b	+	1/1
Bone marrow		b		0/2								b		0/1
Atrophy, testis							△	□			2/4		■	1/1
Reticulum-cell sarcoma					+						1/6			
Lymphosarcoma														
Lymphoma														
Other sarcoma														
Carcinoma														
Lung														
Uterus														
Skin														
Ovary														
Leukemia														
Lymphatic														
Myelogenous														
Fibroadenoma, mammary	+			1/3						+	1/2			
Other benign tumors												+, c		1/2

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Leiomyoma, large bowel.

[d] Fibroma, subcutaneous.

[e] Hemangioma, abdominal lymph node.

Table 12.7b — (Continued)

Dose per treatment	0.5 r, 1000 kv		0.5 r, 250 kv					1 r, 1000 kv			1 r, 250 kv		
Animal No.	28	Freq.	10	39	40	42	Freq.	17	35	Freq.	14	17	Freq.
Sex	M		F	F	F	F		M	F		M	M	
Pulmonary infection					■	□	2/4	■		1/2		□	1/2
Necrosis, liver													
Chronic nephritis			△				1/4						
Nodular periarteritis													
Ulcer													
Small bowel													
Large bowel											△		1/2
Edema, caecal nodes						■	1/4	□		1/2			
Granuloma													
Liver													
Spleen													
Hemorrhage													
Bone marrow	b	0/0										b	0/1
Lymph node													
Adrenal			□			□	2/4				□		1/2
Hyperplasia, splenic pulp	■	1/1	□		△	■	3/4	□		1/2			
Hypoplasia													
Splenic follicles													
Lymph follicles													
Bone marrow	b	0/0										b	0/1
Atrophy, testis								□		1/1	□		1/2
Reticulum-cell sarcoma											+		1/2
Lymphosarcoma													
Lymphoma													
Other sarcoma													
Carcinoma													
Lung													
Uterus													
Skin													
Ovary													
Leukemia													
Lymphatic													
Myelogenous													
Fibroadenoma, mammary				+		+	2/4		+	1/1			
Other benign tumors									+,d	1/2		+,e	1/2

---

[a] The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Leiomyoma, large bowel.

[d] Fibroma, subcutaneous.

[e] Hemangioma, abdominal lymph node.

sections from the sacrificed males exhibited the severe atrophy observed in rats dying in the group exposed at the highest level.

Hypoplasia of the lymph-node follicles was fairly consistent in all those animals sacrificed at the end of treatment; however, similar hypoplasia was noted in the surviving control animals.

Bone-marrow hypoplasia was not evident in these animals nor was any evidence of splenic-follicle hypoplasia found. Hyperplasia of the splenic pulp was consistently present in the control as well as in the treated animals before and after the rest period.

Leukemia did not occur in the animals sacrificed at the termination of the experiment.

Table 12.8—Incidence of Mammary Fibroadenomata in Sacrificed Females

Group	Frequency	Percentage
Control	1/3	33
0.1 r	1/3	33
0.5 r	3/7	43
1 r	3/6	50

Only three instances of malignant tumor were encountered in sacrificed animals. These were reticulum-cell sarcomata distributed as follows: one in an animal in the 0.1 r/day group sacrificed immediately after completion of treatment and one each in the 0.1 and 1 r/day groups of the animals sacrificed 3 months after the last treatment. Of the remainder of the tumors which were benign, mammary fibroadenomata were in preponderance. The identity of the other benign tumors is found in the table footnotes. This distribution did not suggest any relation to exposure to X radiation.

Table 12.8 shows the occurrence of mammary fibroadenomata in sacrificed females, including those sacrificed after 3 months rest, distributed according to dose groups.

Even though the number of animals is small, these findings, in the light of distribution of cases in the group that died during treatment, suggest a relation of the development of mammary fibroadenoma to chronic X radiation.

(c) Animals That Died During Rest Period. Thirty-two rats, including controls, that died during the 3-month rest period were examined histologically. Table 12.9 summarizes the pertinent findings in each.

Table 12.9 — Findings in Rats Dying After 622 Exposures to X Rays[a]

Dose per treatment	Control					0.1 r, 1000 kv						0.1 r, 250 kv				0.5 r, 1000 kv						
Postexposure, weeks (approx.)	1	4	8	9	Freq.	2	3	5	6	13	Freq.	7	9	9	Freq.	1	1	4	5	8	11	Freq.
Animal No.	24	25	39	40		39	42	18	44	29		5	41	34		48	36	41	4	8	47	
Sex	M	M	F	F		M	F	M	F	M		M	F	F		F	F	F	M	F	F	
Pulmonary infection	□	■	■	□	4/4	■	△	■		■	4/5	△	△		2/3	□		△		■		3/6
Necrosis, liver										■	1/5					□		□		□	□	4/6
Chronic nephritis	□		□		2/4	□	△	△			3/5	▲			1/3			□	□			2/6
Nodular periarteritis	■				1/4			△			1/5	△			1/3							
Ulcer																						
Small bowel																				b		0/5
Large bowel																				b		0/5
Edema, caecal nodes	■				1/4			□			1/5											
Granuloma																						
Liver																□	□					2/6
Spleen							□				1/5											
Hemorrhage																						
Bone marrow							b				0/4									b		0/5
Lymph node																	b			b	b	0/3
Adrenal			□		1/4								□		1/3	△	■	■			■	4/6
Hyperplasia, splenic pulp			■	△	2/4	△	■			■	3/5	■	△	△	3/3	△		■		□	△	4/6
Hypoplasia																						
Splenic follicles		□	□	■	3/4		■		△	□	3/5	■	△	□	3/3		□	■	■	▲	■	5/6
Lymph follicles		+	+		2/4	+	+		+	+	4/5		+		1/3		b	+	+	b	b	2/3
Bone marrow							b		■		1/4									b		0/5
Atrophy, testis	▲	△			2/2	■		□			2/3	△			1/1							
Reticulum-cell sarcoma																						
Lymphosarcoma																						
Lymphoma																						
Other sarcoma																						
Carcinoma																						
Kidney																						
Uterus																						
Skin or mammary																						
Ovary																						
Leukemia																						
Lymphatic																						
Myelogenous																						
Fibroadenoma, mammary									+		1/2			+	1/2		+	+				2/5
Other benign tumors													+,d		1/3				+,e			1/6
Miscellaneous									h					i						j	h	

[a] The following symbols have been used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No sections examined.

[c] Fibrosarcoma involving region of caecal lymph nodes.

[d] Fibroma, subcutaneous.

[e] Parathyroid adenoma.

[f] Papillary adenoma, lung.

[g] Islet adenoma, pancreas.

[h] Lack of ovarian follicles.

[i] Moderate pulmonary edema.

[j] Necrosis, renal cortical tubules.

Table 12.9 — (Continued)

Dose per treatment	0.5 r, 250 kv						1 r, 250 kv				1 r, 1000 kv						
Postexposure, weeks (approx.)	1	5	6	8	10	Freq.	3	7	10	Freq.	1	3	4	5	5	12	Freq.
Animal No.	3	38	24	19	22		27	18	22		35	46	10	25	45	2	
Sex	M	F	M	M	M		M	M	M		F	F	F	M	F	M	
Pulmonary infection	■	■	△	□	△	5/5		■	△	2/3	■	□			■	■	4/6
Necrosis, liver		□				1/5		■		1/3							
Chronic nephritis			■	△		2/5						△	△	b	■	■	4/5
Nodular periarteritis																	
Ulcer																	
Small bowel														b			0/5
Large bowel							△			1/3				b			0/5
Edema, caecal nodes							■			1/3							
Granuloma																	
Liver	□					1/5											
Spleen																	
Hemorrhage																	
Bone marrow																	
Lymph node		■				1/5					b						0/5
Adrenal			□			1/5		■		1/3	□	b	□		△		3/5
Hyperplasia, splenic pulp	△	▲	□	□		4/5	■		□	2/3		△		△	▲	□	4/6
Hypoplasia																	
Splenic follicles	□	△	■	▲	■	5/5	■	■	■	3/3	□	■	■		△	△	5/6
Lymph follicles	+	+	+	+	+	5/5		+	+	2/3	b	+	+	+	+	+	5/5
Bone marrow																	
Atrophy, testis	■			□		2/4	△	■		2/3				□		△	2/2
Reticulum-cell sarcoma																	
Lymphosarcoma																	
Lymphoma																	
Other sarcoma														+,c			1/6
Carcinoma																	
Kidney				+		1/5											
Uterus																	
Skin or mammary																	
Ovary																	
Leukemia																	
Lymphatic																	
Myelogenous																	
Fibroadenoma, mammary		+				1/1						+	+		+		3/4
Other benign tumors														+,f		+,g	2/6
Miscellaneous																	

The inflammatory lesions in these animals apparently showed no relation to the X radiation, as a study of Table 12.9 will indicate.

Atrophy of the testis among the male animals of this group was erratic in both frequency and severity. One control animal showed severe atrophy of the testis of a type other than that seen in dying males in the 10 r/day group. The number of males in all groups dying after exposure was so small as to preclude interpretation of these results on the basis of the frequency of occurrence.

In reference to the hematopoietic system in these animals, no appreciable variation from the control animals is apparent. When the various dosage groups and the various groups identified as to time of death are compared, no indication is found that irradiation has influenced these organs. The changes indicated in Table 12.9 were not specific and may have been produced by factors other than irradiation.

Among the animals that died during the 3-month rest period, an apparently significant increase in frequency of adrenal hemorrhage was noted in the 0.5 and 1 r/day groups.

Two malignant tumors were encountered. One was a fibrosarcoma in the region of the caecum of an animal in the 1 r/day group. The second was a carcinoma of the kidney in a rat in the 0.5 r/day group. There was no evidence to indicate that irradiation produced these tumors.

Mammary fibroadenomata were again common in this group of animals. None was found in the control group; two cases were present in the 0.1 r/day group, three in the 0.5 r/day group, and three in the 1 r/day group. These figures may be represented by the following percentages: 0, 50, 50, and 75. As in the sacrificed groups, the number of females present was small. However, the pattern of occurrence of this tumor was similar to that in sacrificed rats and in those dying during treatment and strongly suggested a relation to irradiation.

The other benign tumors were subcutaneous fibroma, parathyroid adenoma, papillary adenoma of the lung, and islet adenoma of the pancreas. The two last-named tumors were present in separate animals in the 1 r/day group. The two former tumors were encountered, one each in the 0.1 and 0.5 r/day groups.

The frequency of occurrence of all pertinent lesions in all groups is presented in summary in Tables 12.10 to 12.14.

1.4 Discussion. (a) Testis. The results of the histological study of the tissues of rats exposed to irradiation under the conditions of this experiment indicate that only in the case of the testis of male animals in the 10 r/day group are there changes significantly frequent or severe to indicate a definite relation to irradiation. If in this group of animals the degenerative changes were erratic as they were in the control and lower-dose groups, no evidence would exist in this experiment to show that this organ was at all affected by irradiation. The results obtained in acute irradiation experiments point to the fact that X radiation affects the most primitive cells and therefore a failure of production at the source occurs. In time, then, it may be ex-

Table 12.10 — Frequencies and Percentages of All Pertinent Lesions in All Rats, Excluding Those at 10 r/Day Level

	Control		0.1 r		0.5 r		1 r	
Lesion	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pulmonary infection	32/47	68	70/97	72.1	59/94	62.7	59/97	60.8
Necrosis, liver	1/46	2.1	14/95	14.7	9/92	9.7	10/98	10.2
Chronic nephritis	6/44	13.6	21/95	22.1	18/92	19.5	23/98	23.4
Nodular periarteritis	2/47	4.3	5/97	5.1	4/96	4.1	6/99	6.0
Ulcer								
Small bowel	1/45	2.2	1/86	1.1	1/79	1.2	1/88	1.1
Large bowel	2/43	4.6	9/90	10	8/86	9.3	14/95	14.7
Edema, caecal nodes	5/47	10.6	16/97	16.4	14/96	14.5	11/99	11.1
Granuloma								
Liver	4/47	8.5	7/95	7.3	18/92	19.5	15/99	15.1
Spleen	3/45	6.6	5/91	5.4	2/91	2.1	3/98	3
Hemorrhage								
Bone marrow	0/45	0	0/91	0	8/91	8.7	5/94	5.3
Lymph node	1/41	2.4	3/86	3.4	8/85	9.4	10/98	10.2
Adrenal	9/38	23.6	18/83	21.6	21/69	30.4	19/74	25.6
Hyperplasia, splenic pulp	27/42	64.2	56/85	65.8	66/89	74.1	65/92	70.6
Hypoplasia								
Splenic follicles	18/42	42.8	39/85	45.8	49/89	55	59/93	63.4
Lymph follicles	23/38	60.5	45/75	60	43/76	56.5	46/70	65.7
Bone marrow	0/44	0	2/86	2.3	7/86	8.1	4/86	4.6
Atrophy, testis	8/21	38	24/50	48	22/49	44.8	21/52	40.3
Reticulum-cell sarcoma	3/47	6.3	12/97	12.3	7/96	7.2	13/99	13.1
Lymphosarcoma	0/47	0	2/97	2	1/96	1	0/99	0
Lymphoma	0/47	0	1/97	1	0/96	0	0/99	0
Other sarcoma	1/47	2.1	0/97	0	1/96	1	5/99	5
Carcinoma								
Lung	1/47	2.1	0/97	0	0/96	0	0/99	0
Uterus	1/24	4.1	0/46	0	1/47	2.1	1/47	2.1
Skin	0/47	0	0/97	0	2/96	2	2/99	2
Ovary	1/24	4.1	0/46	0	0/47	0	1/47	2.1
Kidney							1/99	1
Leukemia								
Lymphatic	0/47	0	1/97	1	2/96	2	1/99	1
Myelogenous	0/47	0	0/97	0	0/96	0	2/99	2
Fibroadenoma, mammary	2/24	8.3	5/46	10.8	7/47	14.8	11/47	23.4
Other benign tumors	2/47	4.1	3/97	3	5/96	5.2	7/99	7

pected that spermatocytes, spermatids, and finally spermatozoa will disappear. The testis sections of control and lower-dose males exhibiting atrophy showed, for the most part, a failure of maturation. Consequently, it is to be expected that the components of the latter stages of maturation will disappear before the spermatogonia are affected. The changes in the testes caused by avitaminoses, infectious diseases, and senility are similar to the findings in these groups of animals.[2–5] It is not possible to state, with the evidence at hand, that

Table 12.11 — Frequencies and Percentages of All Pertinent Lesions in Rats Dying During Treatment

	Control		0.1 r		0.5 r		1 r		10 r	
Lesion	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pulmonary infection	26/35	74	52/71	73	45/69	65	47/77	61	11/47	23
Necrosis, liver	1/34	3	13/69	19	4/67	6	12/78	15	5/48	10
Chronic nephritis	6/32	19	8/69	12	14/67	21	18/79	23	6/46	13
Nodular periarteritis	1/35	3	1/71	1	4/71	6	4/79	5	1/48	2
Ulcer										
Small bowel	1/33	3	1/60	2	1/56	2	1/69	1	2/43	5
Large bowel	2/31	6	7/64	11	8/62	13	12/76	16	18/46	39
Edema, caecal nodes	2/35	6	11/71	15	11/71	15	9/79	11	9/48	19
Granuloma										
Liver	4/35	11	6/69	9	13/67	19	14/79	18	17/48	35
Spleen	3/33	9	4/65	6	2/66	3	3/78	4	7/45	16
Hemorrhage										
Bone marrow	0/34	0	0/67	0	8/69	12	5/78	6	7/46	15
Lymph node	1/29	3	2/63	3	7/63	11	10/79	13	3/43	7
Adrenal	7/26	27	12/57	21	12/46	26	13/54	24	24/32	75
Hyperplasia, splenic pulp	17/30	57	33/59	56	46/64	72	51/72	71	30/50	60
Hypoplasia										
Splenic follicles	15/30	50	33/59	56	39/64	61	51/73	70	37/40	93
Lymph follicles	16/26	62	29/51	57	27/54	50	35/61	57	26/38	68
Bone marrow	0/33	0	1/63	2	7/64	11	4/70	6	8/41	20
Atrophy, testis	6/71	35	20/36	56	20/39	51	14/38	37	21/21	100
Reticulum-cell sarcoma	3/35	9	10/71	14	7/71	10	12/79	15	2/48	4
Lymphosarcoma	0/35	0	2/71	3	1/71	1	0/79	0	0/48	0
Lymphoma	0/35	0	1/71	1	0/71	0	0/79	0	0/48	0
Other sarcoma	1/35	3	0/71	0	1/71	1	4/79	5	1/48	2
Carcinoma										
Lung	1/35	3	0/71	0	0/71	0	0/79	0	0/48	0
Uterus	1/16	6	0/34	0	1/32	3	1/39	3	0/26	0
Skin	0/35	0	0/71	0	2/71	3	2/79	3	0/48	0
Ovary	1/16	6	0/34	0	0/32	0	0/39	0	0/26	0
Leukemia										
Lymphatic	0/35	0	1/71	1	2/71	3	1/79	1	4/48	8
Myelogenous	0/35	0	0/71	0	0/71	0	2/79	3	2/48	4
Fibroadenoma mammary	1/16	6	1/34	3	1/32	3	5/39	13	9/26	35
Other benign tumors	2/35	6	1/71	1	3/71	4	3/79	4	2/48	6

avitaminoses existed in these rats; however, most of these rats did show rather severe infectious processes, and many were old animals at the time of death.

Testicular degeneration of a type not caused by irradiation, as judged by the above criteria, was found in the testis of male rats in

Table 12.12 — Frequencies and Percentages of All Pertinent Lesions in Rats Sacrificed Immediately After 622 Treatments

	Control		0.1 r		0.5 r		1 r	
Lesion	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pulmonary infection	1/5	20	7/10	70	4/9	44	4/7	57
Necrosis, liver	0/5	0	0/10	0	0/9	0	0/7	0
Chronic nephritis	0/5	0	3/10	30	0/9	0	1/7	14.2
Nodular periarteritis	0/5	0	2/10	20	0/9	0	2/7	28.5
Ulcer								
Small bowel	0/5	0	0/10	0	0/9	0	0/7	0
Large bowel	0/5	0	1/10	10	0/9	0	0/7	0
Edema, caecal nodes	1/5	20	1/10	10	2/9	22	0/7	0
Granuloma								
Liver	0/5	0	0/10	0	0/9	0	1/7	14.2
Spleen	0/5	0	0/10	0	0/9	0	0/7	0
Hemorrhage								
Bone marrow	0/5	0	0/10	0	0/8	0	0/4	0
Lymph node	0/5	0	1/9	11	0/9	0	0/7	0
Adrenal	0/5	0	3/10	30	2/7	28.5	1/6	16.6
Hyperplasia, splenic pulp	5/5	100	10/10	100	8/9	88.8	7/7	100
Hypoplasia								
Splenic follicles	0/5	0	0/10	0	0/9	0	0/7	0
Lymph follicles	4/5	80	9/9	100	9/9	100	4/7	57.1
Bone marrow	0/5	0	0/10	0	0/8	0	0/4	0
Atrophy, testis	0/2	0	0/5	0	0/9	0	1/4	25
Reticulum-cell sarcoma	0/5	0	1/10	10	0/9	0	0/7	0
Lymphosarcoma	0/5	0	0/10	0	0/9	0	0/7	0
Lymphoma	0/5	0	0/10	0	0/9	0	0/7	0
Other sarcoma	0/5	0	0/10	0	0/9	0	0/7	0
Carcinoma								
Lung	0/5	0	0/10	0	0/9	0	0/7	0
Uterus	0/5	0	0/5	0	0/5	0	0/3	0
Skin	0/5	0	0/10	0	0/9	0	0/7	0
Ovary	0/5	0	0/5	0	0/5	0	0/3	0
Leukemia								
Lymphatic	0/5	0	0/10	0	0/9	0	0/7	0
Myelogenous	0/5	0	0/10	0	0/9	0	0/7	0
Fibroadenoma, mammary	0/5	0	1/5	20	1/5	20	2/3	66.6
Other benign tumors	0/5	0	0/10	0	1/9	11	0/7	0

the control and lower-dosage groups of all sacrificed animals. These changes were of variable frequency and severity and showed loss of spermatozoa, spermatids, and spermatocytes, thus leading to the conclusion that these changes bore no relation to irradiation. From the above, it seems reasonable to conclude that male rats given 1 r/day or less for 2 years will not develop testicular changes compatible with X-radiation effect.

Table 12.13 — Frequencies and Percentages of All Pertinent Lesions in Rats Dying After 622 Treatments

	Control		0.1 r		0.5 r		1 r	
Lesion	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pulmonary infection	4/4	100	6/8	75	8/11	72.7	6/9	66
Necrosis, liver	0/4	0	1/8	12.5	5/11	45.4	1/9	11
Chronic nephritis	2/4	50	4/8	50	4/11	36.3	4/8	50
Nodular periarteritis	1/4	25	2/8	25	0/11	0	0/9	0
Ulcer								
Small bowel	0/4	0	0/8	0	0/10	0	0/8	0
Large bowel	0/4	0	0/8	0	0/10	0	1/8	12.5
Edema, caecal nodes	1/4	25	1/8	12.5	0/11	0	1/9	11
Granuloma								
Liver	0/4	0	0/8	0	3/11	27.3	0/9	0
Spleen	0/4	0	1/8	12.5	0/11	0	0/9	0
Hemorrhage								
Bone marrow	0/4	0	0/7	0	0/10	0	0/9	0
Lymph node	0/4	0	0/8	0	1/8	12.5	0/8	0
Adrenal	1/4	25	1/8	12.5	5/11	45.5	4/8	50
Hyperplasia, splenic pulp	2/4	50	6/8	75	8/11	72.7	6/9	66
Hypoplasia								
Splenic follicles	3/4	75	6/8	75	10/11	90.9	8/9	89
Lymph follicles	2/4	50	5/8	62.5	7/8	87.5	7/8	87.5
Bone marrow	0/4	0	1/7	14.3	0/10	0	0/9	0
Atrophy, testis	2/2	100	3/4	75	2/5	40	4/5	80
Reticulum-cell sarcoma	0/4	0	0/8	0	0/11	0	0/9	0
Lymphosarcoma	0/4	0	0/8	0	0/11	0	0/9	0
Lymphoma	0/4	0	0/8	0	0/11	0	0/9	0
Other sarcoma	0/4	0	0/8	0	0/11	0	1/9	11
Carcinoma								
Kidney	0/4	0	0/8	0	1/11	9	0/9	0
Uterus	0/2	0	0/4	0	0/6	0	0/4	0
Skin	0/4	0	0/8	0	0/11	0	0/9	0
Ovary	0/2	0	0/4	0	0/6	0	0/4	0
Leukemia								
Lymphatic	0/4	0	0/8	0	0/11	0	0/9	0
Myelogenous	0/4	0	0/8	0	0/11	0	0/9	0
Fibroadenoma, mammary	0/2	0	2/4	50	3/6	50	3/4	75
Other benign tumors	0/4	0	1/8	12.5	1/11	9	2/9	22

There is no evidence to suggest that injury followed by recovery occurred in the testes of the males in the lower-dose groups. The possibility of such occurrence is apparent, but the proof cannot be ascertained from this material.

(b) Hematopoietic System. It is generally known that the hematopoietic system is strongly affected by acute X radiation. However,

Table 12.14 — Frequencies and Percentages of All Pertinent Lesions in Rats Sacrificed 3 Months After Last Treatment

	Control		0.1 r		0.5 r		1 r	
Lesion	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pulmonary infection	1/3	33.3	5/8	62.5	2/5	40	2/4	50
Necrosis, liver	0/3	0	0/8	0	0/5	0	0/4	0
Chronic nephritis	0/3	0	6/8	75	1/5	20	0/4	0
Nodular periarteritis	0/3	0	0/8	0	0/5	0	0/4	0
Ulcer								
Small bowel	0/3	0	0/8	0	0/5	0	0/4	0
Large bowel	0/3	0	1/8	12.5	0/5	0	1/4	25
Edema, caecal nodes	0/3	0	3/8	37.5	1/5	20	1/4	25
Granuloma								
Liver	0/3	0	1/8	12.5	0/5	0	0/4	0
Spleen	0/3	0	0/8	0	0/5	0	0/4	0
Hemorrhage								
Bone marrow	0/2	0	0/7	0	0/4	0	0/3	0
Lymph node	0/3	0	0/7	0	0/5	0	0/4	0
Adrenal	1/3	33.3	2/8	25	2/5	40	1/4	25
Hyperplasia, splenic pulp	3/3	100	7/8	87.5	4/5	80	1/4	25
Hypoplasia								
Splenic follicles	0/3	0	0/8	0	0/5	0	0/4	0
Lymph follicles	1/3	33.3	2/7	28.5	0/5	0	0/4	0
Bone marrow	0/2	0	0/7	0	0/4	0	0/3	0
Atrophy, testis	0/0	0	3/5	60	0/1	0	2/3	66.6
Reticulum-cell sarcoma	0/3	0	1/8	12.5	0/5	0	1/4	25
Lymphosarcoma	0/3	0	0/8	0	0/5	0	0/4	0
Lymphoma	0/3	0	0/8	0	0/5	0	0/4	0
Other sarcoma	0/3	0	0/8	0	0/5	0	0/4	0
Carcinoma								
Lung	0/3	0	0/8	0	0/5	0	0/4	0
Uterus	0/3	0	0/3	0	0/4	0	0/1	0
Skin	0/3	0	0/8	0	0/5	0	0/4	0
Ovary	0/3	0	0/3	0	0/4	0	0/1	0
Leukemia								
Lymphatic	0/3	0	0/8	0	0/5	0	0/4	0
Myelogenous	0/3	0	0/8	0	0/5	0	0/4	0
Fibroadenoma, mammary	1/3	33.3	1/3	33.3	2/4	50	1/1	100
Other benign tumors	0/3	0	1/8	12.5	0/5	0	2/4	50

the animals studied in this experiment showed no marked changes with respect to the blood-forming organs.

Differential cell counts made from femoral bone-marrow smears from the experimental animals did not differ significantly from the control values. This finding lends little weight to any assumption that the mild to moderate hypoplasia of the bone marrow noted in the sections of the bone of the 10 r/day group is significant.

There was an increase in the frequency and degree of hypoplasia in the lymph nodes of the animals that died in the 10 r/day group. These changes occurred in the controls and lower dose levels and were inconsistent in degree and frequency.

With reference to the spleen, the animals in the various groups failed to show any important change, except those in the 10 r/day group. Here the organ showed hypoplasia of the lymphoid follicles more frequently than in the controls. This evidence may be interpreted as being the result of irradiation; however, substantial proof of this fact is lacking.

The sacrificed animals likewise failed to exhibit conclusive evidence of irradiation effect in the hematopoietic system.

(c) Inflammatory Lesions. The two inflammatory lesions having the greatest significance in this experiment were pulmonary infection and the Salmonella syndrome. In the case of the former there was a significant reduction in frequency of the lesion in the 10 r/day group, whereas in the case of the latter there was a significant increase in frequency of the lesion in the same group. The other inflammatory lesions did not create a pattern by means of their frequency or severity. In the sacrificed groups the small number of animals did not permit an accurate evaluation. The frequencies of inflammatory lesions in these groups were erratic.

These findings lack a completely satisfactory explanation. It seems most probable that the apparent reduction in pulmonary infection in the 10 r/day group was due to the high mortality from other causes. The marked increase in salmonellosis suggests a number of possible explanations. The sensitivity of the intestinal mucosa to radiation is well known,[6] and the possibility of the lowering of the resistance of the mucosa to bacterial invasion seems worthy of consideration. On the other hand, the explanation may lie in a more generalized lowering of resistance to infection—the specific type of infection depending upon the type of organisms to which the animals were exposed. In this respect it is of interest that the occurrence of salmonellosis was markedly greater in the rats exposed to 10 r/day with machine B than in those exposed with machine A. The fact that rats were caged in groups according to dosage and machine suggests that the findings may have had some epidemiological basis.

(d) Hemorrhagic Manifestations. Hemorrhagic lesions in this experiment were infrequent. Their occurrence was limited to the bone marrow, lymph nodes, and adrenal. In the two former organs it was difficult to interpret this lesion since its occurrence was erratic. No pattern of frequency or severity was observed. However, the fact that few instances of hemorrhage in the bone marrow or lymph nodes in the control groups occurred suggested the possibility of irradiation as the cause.

In the case of adrenal hemorrhage in animals that died, the findings indicated a more definite pattern. There was an increase in lesion frequency as the dose increased. It appeared in approximately 25 per cent of control animals and for that reason was at first thought to be unimportant. However, the animals that died showed a significantly higher frequency of moderately severe adrenal hemorrhage at the 10 r/day level than in the controls or lower-dose groups. The mechanism for the production of these lesions is obscure. The sacrificed treated animals showed an increase in frequency of the lesion over the control group, but the percentages did not parallel the increase in dose. It should be remembered, however, that the number of animals in these groups was small.

Although the evidence here submitted suggests, at least on the surface, that irradiation contributed to the appearance of this lesion, the fact remains that further experimentation is necessary to establish or refute this relation.

(e) Tumors. Twelve rats that died during treatment showed either myelogenous or lymphatic leukemia. There were no instances of the disease in the sacrificed or control groups. The frequency of the disease in the various dosage groups increased with the number of roentgens per day administered. These findings suggest that leukemia may develop as a result of irradiation of 0.1 r/day or higher, or that the onset of the disease may be caused to occur earlier than would otherwise be the case.

Tumors other than leukemias may be classified in a general way as follows:

Sarcomata	Carcinomata	Benign tumors
Reticulum cell	Lung	Fibroma
Osteogenic	Ovary	Papillary cystadenoma (ovary)
Sarcoma (origin unknown)	Uterus	Adenoma of liver
Lymphoma	Skin	Adenoma of testis
Lymphosarcoma	Mammary	Fibroadenoma of mammary gland
	Kidney	

Excluding those originating in the mammary gland, none of the above-listed tumors, individually or as a group, were significantly increased in the irradiated animals. Table 12.15 shows a comparison of control and treated animals with respect to benign and malignant tumors and illustrates this statement.

Table 12.16 illustrates the increase of incidence of mammary fibroadenomata with increasing doses of irradiation. Only females were considered for this table.

The significance of the increased incidence gains importance in view of the fact that the group with the shortest survival time developed the largest percentage of such tumors.

Table 12.15 — Incidence of Tumors Other than Leukemia and Mammary Fibroadenoma

Dosage group	Control		0.1 r		0.5 r		1.0 r		10.0 r	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Malignant tumors, excluding leukemia	7/47	15	15/97	15.4	12/96	12.5	23/99	23	3/47	6.4
Benign tumors, excluding mammary fibroadenoma	2/47	4.2	3/97	3.1	5/96	5.2	7/99	7	2/47	4.3
Total tumors, excluding leukemia and mammary fibroadenoma	9/47	19.2	18/97	18.5	17/96	17.7	30/99	30	5/47	10.7

Table 12.16 — Incidence of Benign Mammary Tumors in Female Rats

Dose, r	Died during irradiation	Sacrificed at end of irradiation	Died during rest period	Sacrificed following rest period	Total	
					Frequency	%
0.1	1/34	1/5	2/4	1/3	5/46	10.8
0.5	1/32	1/5	3/6	2/4	7/47	14.8
1.0	5/39	2/3	3/4	1/1	11/47	23.4
10.0	9/26				9/26	34.6
Control	1/16	0/3	0/2	1/3	2/24	8.3

Consequently the evidence obtained indicates that there is, at least, an implied relation between the increased frequency of mammary fibroadenomata and irradiation. A similar relation exists with respect to leukemias. For further evidence of the relation between irradiation and leukemia and neoplasms in general, the reader is referred to Chap. 2.

(f) Causes of Death. The probable causes of death for all the animals that died during this experiment may be assigned to the following diseases or lesions in approximately 70 per cent of the cases: pulmonary infection, malignant tumors, chronic nephritis, Salmonella infection, and liver necrosis. No cause of death could be assigned in approximately 30 per cent of the animals. In the 10 r/day group, where all animals died before the end of the irradiation period, the most frequent lesions were ulceration of the bowel and leukemia. These two lesions were present in 66 per cent of this group of animals. An additional 4 per cent of the group had other malignant tumors. No specific change was encountered which could be definitely identified with irradiation except the testicular change. The latter has provided the only consistent lesion referable to irradiation.

Table 12.17 — Frequencies and Percentages of Common Lesions Possibly Related to Cause of Death of Rats That Died During the Experiment*

Dose group	Exposure deaths		Postexposure deaths		Exposure deaths		Postexposure deaths	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%
	Malignant Tumor				Pulmonary Infection			
Control	6/35	17.1	0/4	0	21/35	60	2/4	50
0.1 r	14/71	19.7	0/8	0	42/71	59.1	6/8	75
0.5 r	14/71	19.7	1/11	9	36/69	52.1	6/11	54.5
1.0 r	22/79	27.8	1/9	11.1	36/77	46.7	5/9	55.5
10.0 r	9/48	18.7	None		8/47	17	None	
	Salmonella Infection				Chronic Nephritis			
Control	7/35	20	0/4	0	4/32	12.5	0/4	0
0.1 r	13/71	18.3	0/8	0	7/69	10.1	3/8	37.5
0.5 r	14/71	19.7	0/11	0	11/67	16.4	2/11	18.1
1.0 r	20/77	25.9	1/9	11.1	13/79	16.4	4/8	50
10.0 r	19/48	39.5	None		5/46	10.8	None	
	Liver Necrosis							
Control	0/34	0	0/4	0				
0.1 r	9/69	13	1/8	12.5				
0.5 r	2/67	2.9	0/11	0				
1.0 r	7/78	8.9	1/9	11.1				
10.0 r	3/48	6.2	None					

*Mild changes were not considered in compiling the above figures, thus accounting for the discrepancies in the results between this table and that showing total frequencies and percentages for all lesions at the various doses.

Consequently it cannot be said, without fear of criticism, that any of the animals died as a direct result of irradiation. At most, there can only be an indirect relation through the possibility of increasing the frequency or severity of intercurrent infection or the production of mammary tumors and leukemias.

Table 12.17 summarizes the apparent causes of death and the incidence of each for all animals that died during the experiment, including those that died after the termination of treatment. Mild forms of the lesions mentioned were not considered as the possible causes of death. Only those lesions of moderate to marked severity were included. Another table is used to indicate the frequency and percentage of all pertinent lesions for all animals regardless of severity.

1.5 Conclusions. The following generalizations seem warranted after histological examination of chronically X-radiated rat tissues:

1. Specific histological criteria of chronic X-radiation injury are lacking in this experiment.
2. The testis is severely injured with administration of 10 r/day. The earliest time of occurrence of this injury is not apparent. The testis is not specifically injured at 0.1, 0.5, or 1.0 r/day.
3. There is evidence to suggest that the incidence of leukemia in all treated animals and mammary fibroadenomata in females is increased by chronic X radiation. The need for further experimentation with respect to these tumors and their relation to chronic X radiation is apparent.
4. The changes in the hematopoietic system exclusive of the leukemias are not impressive.
5. Deaths cannot be explained in approximately 30 per cent of the animals that died during the experiment. Various diseases and lesions, none of which are directly related to irradiation, apparently caused the death of approximately 70 per cent.
6. There was no histological evidence of X-radiation injury in the heart, lung, liver, kidney, bowel, pancreas, thyroid, trachea, skin, bone, or ovary.
7. The histological changes were not influenced by the voltages used in this experiment.

2. FINDINGS IN RABBITS

2.1 Introduction. Like the study in rats, the study of rabbits exposed to X radiation in doses of 0.1, 0.5, 1.0, and 10.0 r/day for 1 year (310 treatments) was divided into groups based on the time and manner of death. These groups were as follows: rabbits that died during the treatment; rabbits sacrificed at the end of 310 treatments; and rabbits sacrificed at the end of a 3-month rest following treatment. Separate groups of animals were treated with 250- and 1000-kv machines at the various doses. However, experience has taught that no histological differences occur which are attributable to voltage; therefore all animals receiving a given dose were considered together, irrespective of voltage. The number of animals in each of the three groups receiving the various dosages are listed in Tables 12.18 to 12.20.

The methods used in this study were identical with the methods used in the study of rats.

2.2 Results. (a) Rabbits That Died During Treatment. Table 12.21 summarizes the pertinent lesions, their degree of intensity (on a basis of trace to severe injury), and their frequency. It will be noted that the number of lesions appearing in the list is considerably smaller than the list in the tables used for the rats.

Table 12.18—Rabbits That Died During Treatment

	Dose, r/day				
	0.1	0.5	1.0	10.0	Controls
Males	5	3	1	5	4
Females	4	4	6	5	1
Total	9	7	7	10	5

Table 12.19—Rabbits Sacrificed After 310 Treatments

	Dose, r/day				
	0.1	0.5	1.0	10.0	Controls
Males	2	2	2	2	2
Females	2	2	3	2	1
Total	4	4	5	4	3

Table 12.20—Rabbits Sacrificed After 310 Treatments and a 3-month Rest

	Dose, r/day				
	0.1	0.5	1.0	10.0	Controls
Males	2	3	7	2	5
Females	3	4	2	2	2
Total	5	7	9	4	7

The inflammatory lesions are considered to be incidental findings bearing no relation to irradiation. The changes in the hematopoietic system are considered in a similar manner except for bone-marrow hypoplasia. In the latter instance, 5 of 10 animals in the 10 r/day dose level exhibited this lesion.

Table 12.21 — Findings in Rabbits Dying After Exposure to X Rays[a]

Dose per treatment	Control						0.1 r, 1000 kv				0.1 r, 250 kv							0.5 r, 1000 kv			
No. of treatments	54	59	163	265	307		13	259	260		64	108	123	131	242	265		16	57	294	
Animal No.	11	1	15	5	2	Frequency	3	5	10	Frequency	3	2	7	9	1	6	Frequency	4	10	5	Frequency
Sex	M	M	F	M	M		M	M	F		M	M	F	F	M	F		M	F	M	
Pulmonary infection												■					1/6		□		1/3
Pulmonary edema			■			1/5					■				■		2/6		■		1/3
Liver																					
Necrosis															■		1/6		■	■	2/3
Parasites					□	1/5			□	1/3			■		■	■	3/6				
Chronic nephritis				△		1/5		■		1/3			■		△	△	3/6			△	1/3
Hemorrhage, bone marrow	□					1/5		□	□	2/3											
Hyperplasia, splenic pulp				■	□	2/5						△					1/6				
Hypoplasia																					
Splenic follicles				□	□	2/5	□	□		2/3		■					1/6				
Lymph nodes		b		+	+	2/4		+		1/3	+	+	+	+	+	+	6/6			+	1/3
Bone marrow				□	■	2/5	□			1/3										■	1/3
Atrophy, testis				b	■	1/3		■		1/2	■	□					2/3	□		c	1/1
Miscellaneous[e]	f	g																			

[a] The following symbols are used to denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Testis immature.

[d] Spermatogonia, spermatocytes principally affected.

[e] There were no tumors observed.

[f] Hemorrhage in stomach moderately severe.

[g] Pulmonary hemorrhage, mild.

[h] Ulceration, large bowel.

[i] Septicemia.

[j] Ulceration and hemorrhage, small bowel.

Table 12.21 — (Continued)

Dose per treatment	0.5 r, 250 kv					1 r, 1000 kv						1 r, 250 kv			10 r, 1000 kv							10 r, 250 kv				
No. of treatments	47	87	93	171		32	41	42	60	222		159	163		32	173	177	226	255	291		43	57	139	144	
Animal No.	6	1	8	10	Frequency	6	7	8	3	10	Frequency	10	9	Frequency	1	7	3	4	9	2	Frequency	9	5	10	8	Frequency
Sex	F	M	F	F		F	F	F	M	F		F	F		M	F	M	M	F	M		F	M	F	F	
Pulmonary infection		■	△	△	3/4	■				■	2/5	△	□	2/2	□		△		△	△	4/6		□			1/4
Pulmonary edema	■				1/4		■				1/5															
Liver necrosis																										
Necrosis							b		■		1/4						■				1/6					
Parasites		■			1/4		b		■		1/4	△		1/2											■	1/4
Chronic nephritis							▲				1/5	△	△	2/2												
Hemorrhage																										
Bone marrow		b			0/3																					
Hyperplasia,																										
Splenic pulp										▲	1/5				b			■	□		2/5			△		1/4
Hypoplasia,																										
Splenic follicles										■	1/5					■	■		■		3/6		△	■		2/4
Lymph nodes	+	+	b		2/3	+	b		+	b	2/3		+	1/2	b	+	+	+	b	+	4/4	b	b	+	+	2/2
Bone marrow		b			0/3										■				□	■	3/6			■	□	2/4
Atrophy, testis		c							△		1/1				△,d		△	▲		▲	4/4		▲			1/4
Miscellaneous	f												h		i			g				g			j	

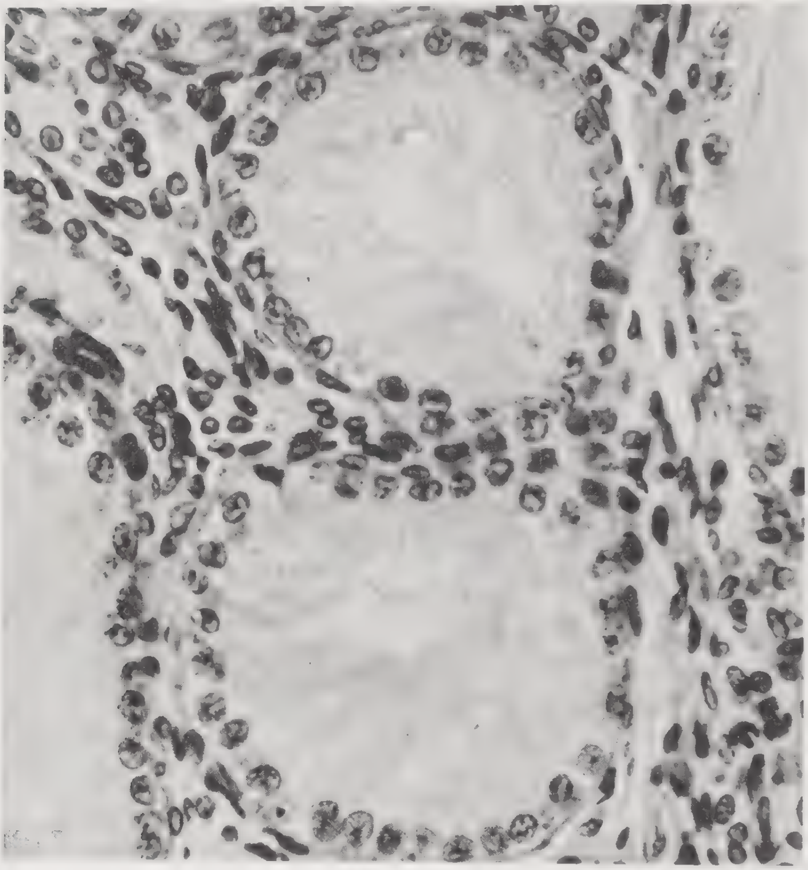

Fig. 12.8—Section of testis from rabbit A4 which died after 226 treatments at 10 r per treatment. The seminiferous tubules are reduced in size and contain only Sertoli cells. 440×

In the control and the 0.1 and 0.5 r/day groups, the changes noted in the testes of an occasional male animal in each group were of a type not considered to be compatible with irradiation effect. One male animal in the 0.5 r/day group showed immature testes.

The testes of the five male rabbits at the 10 r/day level showed changes considered to be caused by X radiation. Figure 12.8 shows the type of testicular change observed. Animal A1 exhibited incomplete damage to the spermatogenic elements, but the injury was represented by a loss of spermatogonia and spermatocytes, a type of change considered to be characteristic of the early effects of irradiation (see Fig. 12.9). This animal died at the end of the first month after a total of 320 r had been administered. Animal A5 showed complete absence of germinal cells after about 2 months of treatment. The observations on these two rabbits suggest that testicular damage in chronic irradiation may occur early.

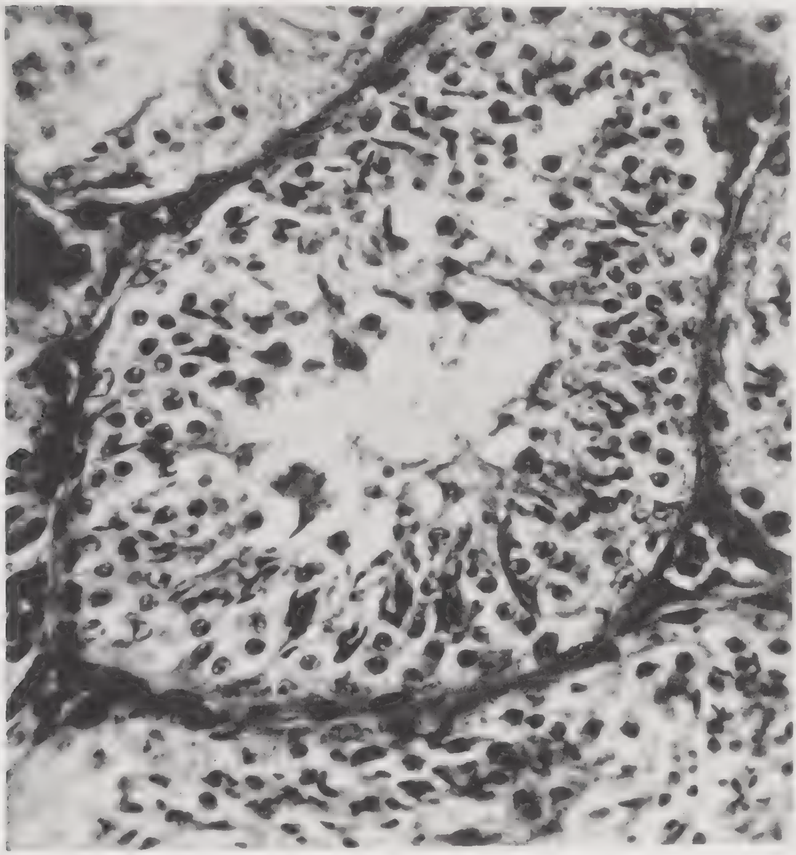

Fig. 12.9—Section of testis from rabbit A1 which died after only 32 treatments at 10 r per treatment, showing several representative tubules. In general the more immature cells show the greatest reduction in number. 440×

A single male (A3) in the 1 r/day group exhibited rather marked testicular changes characterized by complete depletion of spermatozoa and spermatids with marked reduction in the number of spermatocytes and spermatogonia (see Fig. 12.10). There were, however, moderate numbers of spermatozoa in the distal epididymis. This rabbit died at the end of 2 months of treatment after having received a total of 60 r.

A normal testis from a control rabbit is shown in Fig. 12.11.

Tumors did not occur in these animals.

(b) Rabbits Sacrificed at Termination of Exposure. Two males and two females were sacrificed in each of the four dose groups. There were two males and one female in the control group. Of these animals, only the two males in the 10 r/day group showed changes which could be associated with irradiation treatment. A complete disappearance of the spermatogenic elements and spermatozoa was en-

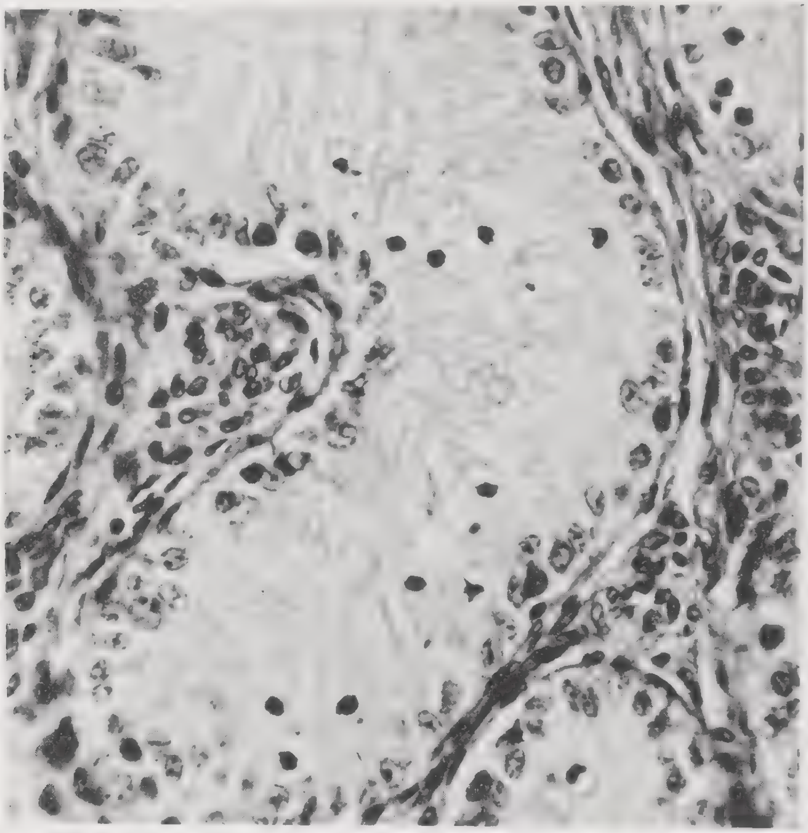

Fig. 12.10—Section of testis from rabbit A3 which died after 60 treatments at 1 r per treatment. The seminiferous tubules contain only Sertoli cells and a few spermatogonia and spermatocytes. The latter show nuclear pycnosis. 440×

countered. Other changes were considered as incidental and without relation to irradiation. Table 12.22 summarizes the lesions.

(c) Rabbits Sacrificed 3 Months After Termination of Exposure. These animals were allowed 3 months rest following cessation of X-ray exposure. The paucity of lesions in the group sacrificed at exactly 1 year suggested that only sections of spleen, testis, ovary, lymph nodes, and bone marrow would be important. Consequently only these sections were taken and studied histologically.

The pertinent findings were so few as to render the use of a summary table unnecessary. All sections were normal except for pneumonia in one female in the 0.5-r group, four males in the 1-r group, and one male in the control group. There was also severe testicular atrophy in both males in the 10 r/day group. Another interesting finding was the lack of follicles in the ovaries of the two females in the 10 r/day group. The tubular degeneration in the testes of the 10

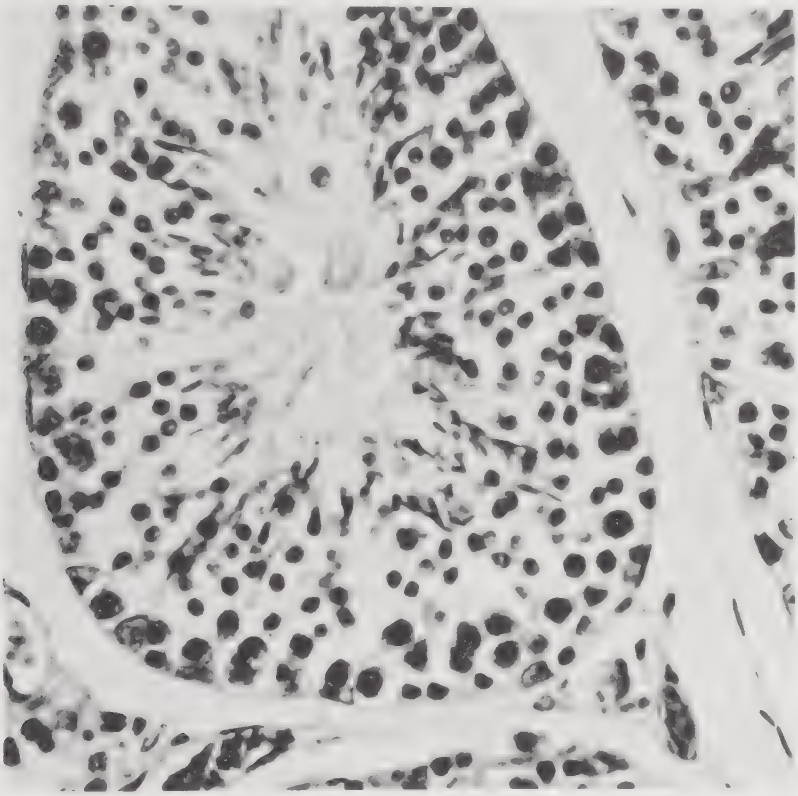

Fig. 12.11—Section of testis from a control rabbit, showing essentially normal seminiferous tubules. 440×

r/day males was severe and of the type observed in this and other species exposed at this level. The testicular atrophy noted in the males of other dose groups was mild and localized and exhibited the greatest injury involving the spermatids and spermatozoa.

2.3 Discussion. In this experiment, as in the experiment with rats, the only consistent lesion that was apparently directly related to irradiation was the testicular injury in the rabbits in the 10 r/day group. The differences in the testicular injury of rabbits in the three lower-dose groups when compared to testes injury of the 10 r/day animals has already been discussed in the section on rats. Bone-marrow hypoplasia of a moderate grade in five rabbits at the 10 r/day level and the lack of follicles in the ovaries of two females at this level are suggestive of irradiation injury.

Neoplastic diseases were not encountered in these animals.

With reference to the time of occurrence of the earliest testicular injury, the findings in animal 1 in the 10 r/day group (died after 32 treatments) suggest that, at this level, testicular atrophy occurs within the first 2 months of repeated daily exposure. Furthermore the type of change observed is compatible with the view that testicular changes from chronic irradiation occur in the same manner as those following a single exposure. Further support of this contention is to be found in the case of one rat exposed to 1.7 n/day (see Chap. 17) in which the testicular atrophy was incomplete, and the principal changes were in the least mature germinal cells.

Table 12.22 — Findings in Sacrificed Chronically X-radiated Rabbits After 310 Treatments*

Dose per treatment	Control			0.1 r, 1000 kv		0.1 r, 250 kv		0.5 r, 1000 kv		0.5 r, 250 kv		1.0 r, 1000 kv		1.0 r, 250 kv			10.0 r, 1000 kv		10.0 r, 250 kv	
Animal No.	4	10	12	1	7	5	10	1	7	3	7	2	9	10	5	7	5	6	2	7
Sex	M	M	F	M	F	M	F	M	F	M	F	M	F	F	M	F	M	F	M	F
Pulmonary infection									■											
Liver inflammation	□	□				□		□	□						□	□		□	□	□
Interstitial nephritis	□	□											□				□	■	□	■
Kidney, active regeneration															□			■	□	
Testis																				
Decrease in spermatogonia																	▲		▲	
Decrease in spermatocytes															□		▲		▲	
Aspermia																	▲		▲	
Aspermia epididymis																	▲		▲	

*The following symbols are used to denote varying degrees of injury: □, mild; ■, moderate; △, moderately severe; and ▲, severe.

Conclusive evidence that X radiation, at the rate of 1 r/day, affects the male germinal epithelium is lacking in this experiment. One point in this connection, however, is of some interest. One rabbit, which died after 60 r had been administered at the rate of 1 r/day, showed marked though incomplete depletion of the germinal cells. The remaining cells were, for the most part, spermatocytes. The fact that the distal part of the epididymis contained mature spermatozoa indicates that the testicular damage had occurred relatively recently — probably within the period of irradiation. These observations certainly point to the exposure to X rays as the cause of the atrophy in this case even though no animals from this group examined at a later date exhibited more than very mild testicular change which was localized to scattered small areas in the organ. This latter type of abnormality has been observed so frequently in nonirradiated animals that no significance can be attached to it.

2.4 Conclusions. 1. The only consistent lesion attributable to X radiation is testicular injury in the 10 r/day groups. Evidence that 1 r/day may damage the testes of rabbits is inconclusive.

2. Ovarian follicles may disappear when doses of 10 r/day are administered for a sufficient length of time.

3. The hematopoietic system in rabbits is not affected to a detectable degree by chronic X radiation under the conditions of this experiment, with the possible exception of the bone marrow in animals exposed at the rate of 10 r/day.

3. FINDINGS IN DOGS

3.1 Introduction. Dogs were exposed at the four different dosage levels used in the preceding experiments and at two additional levels not used in the case of rabbits and rats. The additional groups were given 3 and 6 r/day. Thus the dosage levels were 0.1, 0.5, 1.0, 3.0, 6.0, and 10.0 r/day. Those animals in the three lower-dosage groups were given 622 treatments; those in the three higher-dosage groups received 500 treatments.

The scheduled time of sacrifice and the time of death of certain animals in various dosage groups resulted in further grouping as follows: animals that died during the exposure period; those sacrificed at the end of 622 daily treatments; those sacrificed at the end of a 3-month rest after 622 treatments; and those sacrificed at the end of 500 daily treatments without benefit of rest. Two dogs died during the rest period. These animals were in the 0.1 r/day group. The number of animals in each of these groups is shown in Table 12.23.

Nineteen dogs were permitted to survive indefinitely for other studies and were not available for study here.

3.2 Methods. The dogs that died during the course of exposure were autopsied as soon after death as possible. The sacrificed dogs were killed with an overdose of nembutal given intravenously. Tissue was taken from the heart, lungs, liver, spleen, pancreas, stomach, small bowel, large bowel, adrenal, kidney, lymph nodes, thyroid, ovary, testis, and bone from a vertebra and femur. Smears were taken from femoral bone marrow. Tissue blocks were fixed in Zenker's or Bouin's fluid, embedded in paraffin, and cut into sections 7 μ thick. Sections were stained with Harris' hematoxylin and eosin. Bone-marrow smears were stained by Wright's method.

Tables listing pertinent lesions similar to those used in the histological study of rat and rabbit tissues were used in the study of dog tissues. For an explanation of terms used in the tables, refer to Sec. 1.2 on chronically X-radiated rats.

3.3 Results. (a) Dogs That Died During Treatment. The findings are summarized in Table 12.24. The dogs are not separated here with regard to the voltage of the X-ray machines since no histological difference could be made between the two groups. These animals did not suffer to an appreciable degree from pulmonary infection. In those dogs in which it occurred, it was of a mild to moderate grade. Control dog 1 died of distemper at an early date in the experiment and showed pulmonary infection of a moderately advanced grade.

Table 12.23 — Distribution of Animals According to Dose, Number of Treatments, Sex, and Time of Death*

Dose, r/day	No. of treatments	Died during exposure		Sacrificed at end of exposure		Died during rest period		Sacrificed at end of 3 months rest		Total	Total no. of animals
		M	F	M	F	M	F	M	F		
Control		0	1	0	4	†	†	†	†	5	9
0.1	622	0	1	2	4	0	2	0	7	16	20
0.5	622	0	0	2	4	0	0	0	9	15	20
1.0	622	2	1	3	3	0	0	0	6	15	20
3.0	500	3	0	2	4	0	0	0	0	9	10
6.0	500	3	2	3	2	†	†	†	†	10	10
10.0	500	8	8	2	2	†	†	†	†	20	20
Total		16	13	14	23	0	2	0	22	90	109

*Nineteen animals were permitted to survive indefinitely for other studies, thus accounting for the discrepancy between the totals in the last two columns of the table.

† None remaining.

Liver necrosis occurred in three animals, one of which was the dog that succumbed to distemper. The remaining two were in the 10 r/day groups. An explanation for the liver necrosis in these two animals is not apparent. The virus disease may have caused it in the control animal.

Liver degeneration occurred in four animals, three of which were in the 10 r/day groups. The other example was in the 6 r/day group.

Septicemia appeared in two dogs in the 10 r/day group. The infection was determined by histological examination of the tissue sections and was without bacteriological confirmation. It probably represents terminal blood-stream invasion.

Lymph-node hemorrhage (Fig. 12.12) reached its greatest frequency and intensity in the 10 r/day group. Lymph-node sections were absent in the tissue sections of two dogs in the 10 r/day group. Nine of fourteen dogs examined for lymph-node hemorrhage showed this lesion. The degree of hemorrhage was moderately severe or severe in all but one animal, the section of which indicated a moderate degree. In other groups, only three instances of mild or moderate

Table 12.24 — Findings in Dogs Dying After Exposure to X Rays[a]

Dose per treatment	Control		0.1 r, 250 kv	1 r, 1000 kv		1 r, 250 kv	3 r, 1000 kv	3 r, 250 kv		6 r, 1000 kv				6 r, 250 kv	10 r, 1000 kv							10 r, 250 kv								
No. of treatments	21	551	603	533	551	513	393	421	435	344	369	384	491	218	120	124	134	183	254	311	359	107	110	115	127	130	132	144	187	266
Animal No.	4	10	1	3	10	9	1	2	1	3	4	2	1	4	1	6	3	8	4	2	5	7	3	9	4	1	2	5	10	8
Sex	M	F	F	M	F	M	M	M	M	M	M	M	F	F	F	M	F	M	F	F	M	M	F	M	F	M	F	F	M	M
Pulmonary infection	△													■					□	□				■					■	
Liver necrosis																														
Necrosis	■																△													■
Degeneration													■								■	■						■		
Septicemia																	+	+												
Hemorrhage																														
Lymph node			□	□									■			▲		▲		□		▲		▲	▲	b	△	b	△	△
Bowel	+			+											+							+	+	+	+		+		+	
Pulmonary					□	■																		■			□		■	
Bladder						▲																								
Bone marrow																						□				■	■			
Atrophy, testis	c			▲		▲	▲	▲	▲	▲	▲	▲				▲		▲			▲	▲		▲		▲			▲	▲
Hypoplasia																														
Lymph node	d	■		■	■		■			■	■	□	□		△	■	△	□				■	□	△	△	b	△	b	□	■
Spleen	□	■	■	■	■	b	□	□		□	▲	■		□	□	△	□	□		■	□			□	■	■	■	△		□
Bone marrow				□											□	△		▲		■		■	■	△	▲	▲	△	△	▲	■
Decreased megakaryocytes															+	+		+		+	+	+	+	+	+	+	+	+	+	+
Miscellaneous													e						f											
Blood counts																														
Red blood cells			7.2	5.6	2.7	5.3	6.6	7.0	5.7	2.1	4.0	2.8		1.9	4.5	3.4	4.6	1.6	2.0	5.8	1.3	1.4	3.4	1.3	3.6	3.1	3.6	1.0	2.0	0.8
White blood cells			4.25	4.6	5.15	8.2	2.45	10.3	8.3	2.8	1.5	4.0		0.5	6.45	1.0	1.4	0.55	129.2	8.5	4.3	3.0	1.35	0.62	1.55	2.1	2.85	2.1	1.33	1.77
Platelets			1.2	1.8	2.9	2.1	1.0	2.1	1.6	0.6	0.3	1.2		0.6	1.6	1.0	1.6	0.1	0.6	0.7	1.2	0.3	0.4	0.6	0.3	0.2	1.5	1.4	0.8	1.0

[a] The following symbols are used to denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Immature.

[d] Lymph-node necrosis.

[e] Possible leukemia.

[f] Myelogenous leukemia.

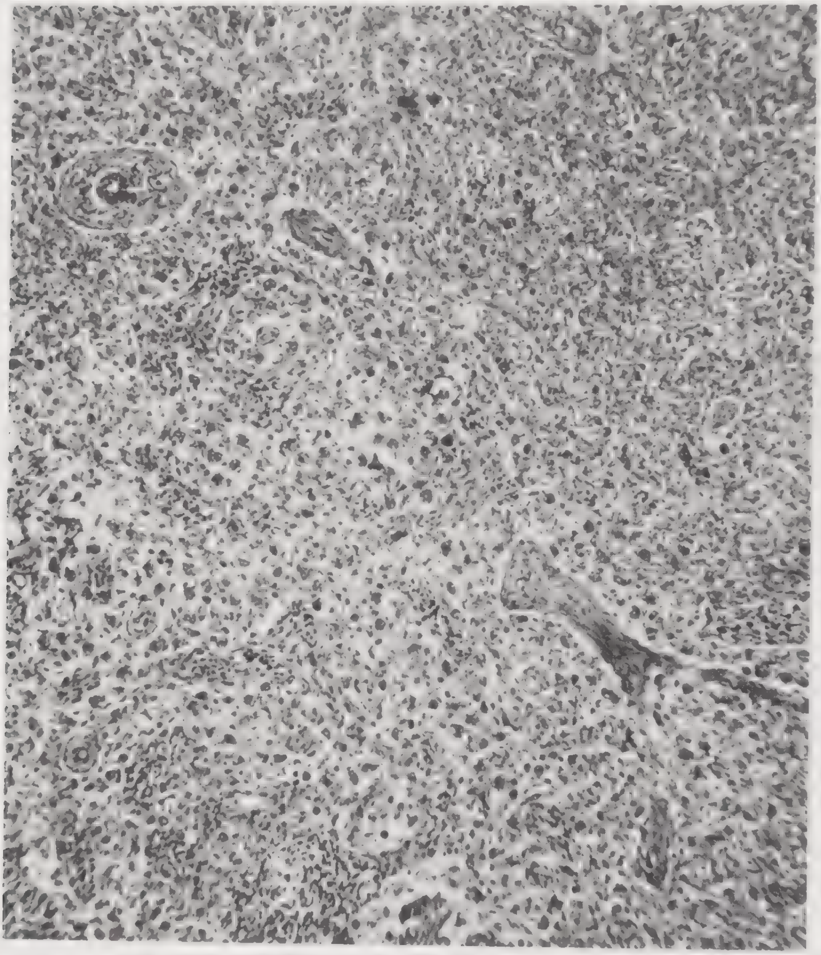

Fig. 12.12—Section of lymph node from dog B9 which died after 115 treatments at 10 r per treatment, showing atrophy of the lymph cords and accumulations of blood and blood pigments in the dilated sinuses. 100×

grades of hemorrhage were encountered. These were not considered to be of significance.

Bowel hemorrhage similarly appeared in its greatest frequency in the 10 r/day group. Seven of sixteen animals in this group exhibited the lesion. Only two instances of a similar lesion were encountered in other groups. One of these was in the dog that died of distemper. The other instance was in an animal in the 1 r/day group.

Pulmonary hemorrhage was infrequent, scattered, and of mild to moderate grade. No significance is attached to this lesion.

A single instance of urinary-bladder hemorrhage of severe grade was encountered in a dog in the 1 r/day group (Fig. 12.13).

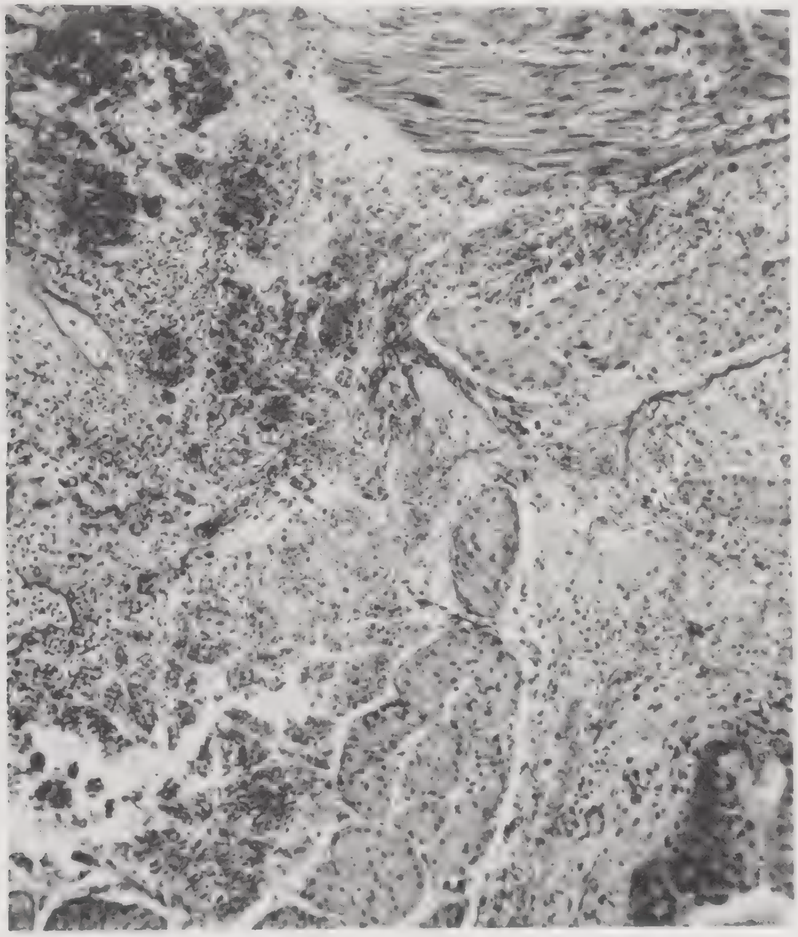

Fig. 12.13—Section of urinary bladder from dog B9 which died after 513 treatments at 1 r per treatment, showing marked hemorrhage in the bladder wall. 100×

Bone-marrow hemorrhage and hyperemia were noted in only three dogs, all of which were in the 10 r/day group. These instances were of mild to moderate grade.

All male dogs from the 1, 3, 6, and 10 r/day groups that died during the exposure period showed severe tubular degeneration of the testes. Germinal elements as well as mature spermatozoa were completely lacking. The type of change observed is shown in Figs. 12.14 to 12.17. The single male control dog, as previously mentioned, died after 3 weeks. Its testes were found to be immature. There was no control material for comparison in this experiment with respect to the testes. However, the results were such that there was little room for doubt

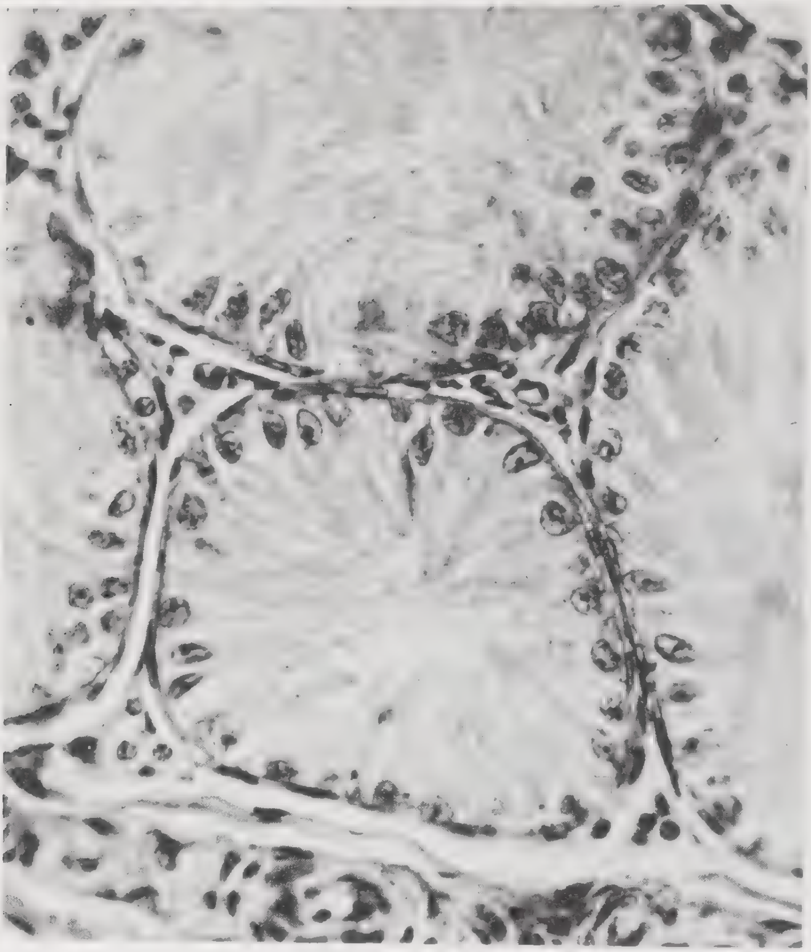

Fig. 12.14—Seminiferous tubules from dog B9 exposed at the rate of 1 r/day and which died after 513 exposures. The tubules contain no germinal elements and are considerably reduced in size. 440×

that the testicular injury was related to irradiation, especially when viewed in the light of the findings in rats and rabbits.

Lymph-node hypoplasia was frequently observed in the animals that died (Fig. 12.12). In the dosage groups below 10 r/day, it was of mild to moderate grade. In the 10 r/day group, it was mild to moderately severe. In two instances in the 10 r/day group, lymph nodes were not present for histological study.

Hypoplasia of the spleen was similarly a common finding in all groups. A single instance of severe grade was encountered in a dog in the 6 r/day group. The remaining examples were of mild to moderate severity.

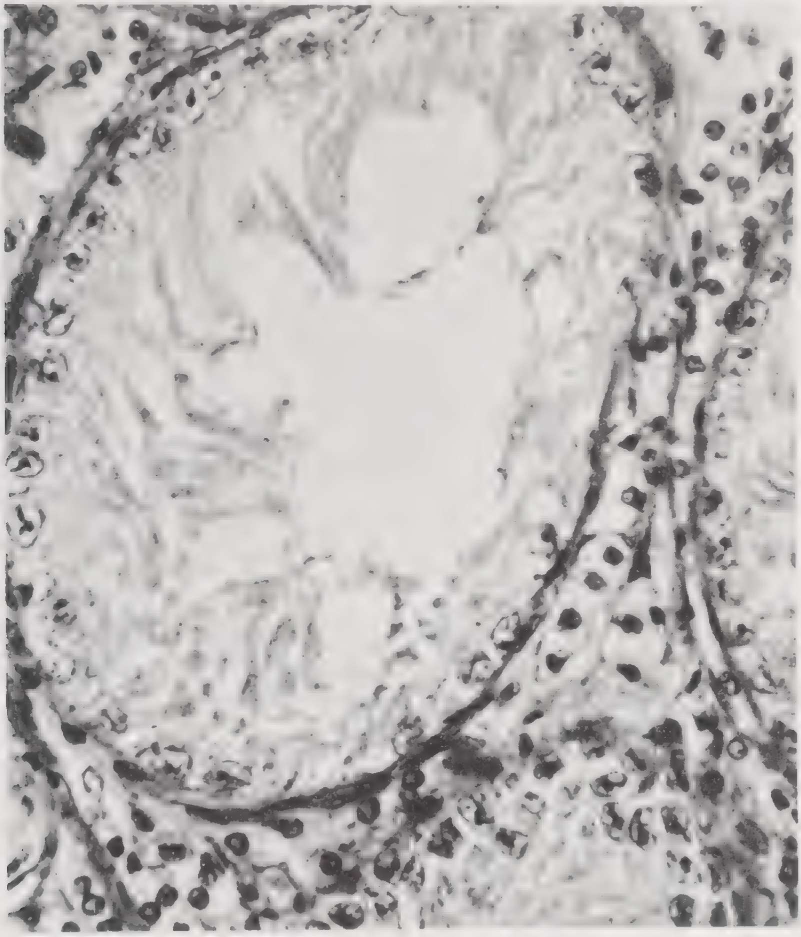

Fig. 12.15—Testis from dog A1 exposed at the rate of 3 r/day (died after 435 exposures). The seminiferous tubules are devoid of germinal cells and spermatozoa. 440×

Hypoplasia of the bone marrow (Fig. 12.18) was by far more common in the 10 r/day group than in any other group. At the 10 r/day level, 13 of 16 dogs (81.25 per cent) showed this lesion. The lesion was characterized by a reduction in the number of parenchymal cells of the marrow. Those cells were of both the erythropoietic and the granulocytopoietic types. The supporting cells apparently were not materially affected. There were four instances of severe hypoplasia in this group. The remaining animals showed the lesion in a moderate to moderately severe degree. A single instance of bone-marrow hypoplasia of mild grade was present in the 1 r/day group.

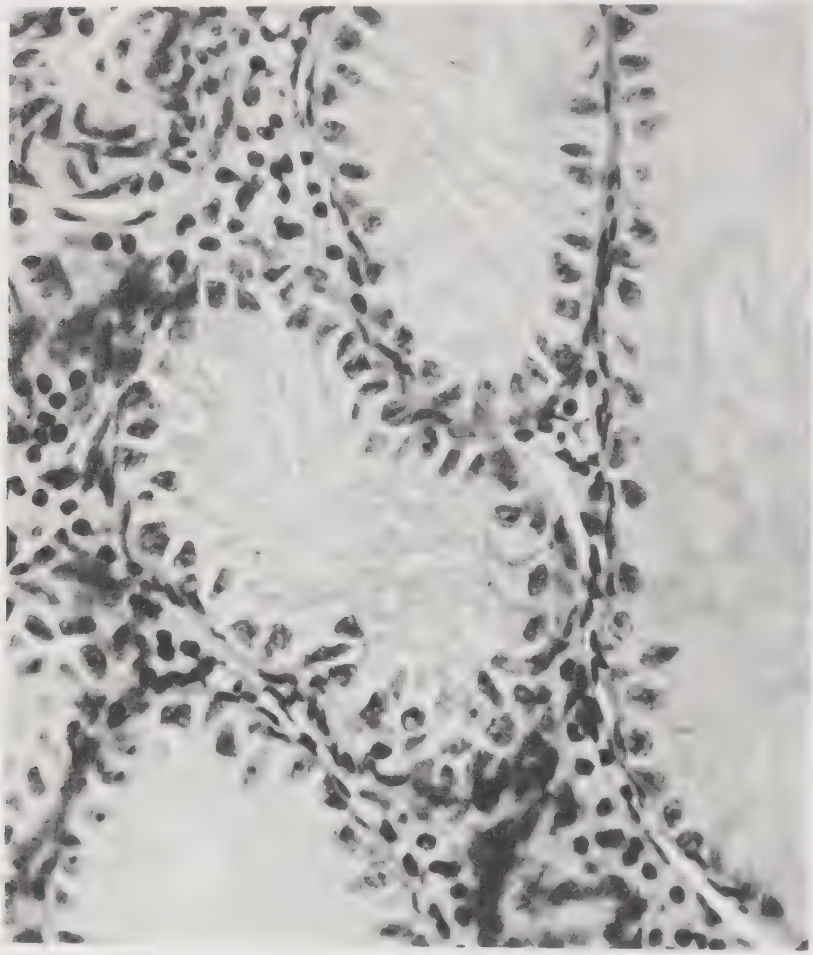

Fig. 12.16—Testicular changes similar to those shown in Figs. 12.14 and 12.15. The dog from which this section was made died after 384 treatments at 6 r per treatment 440×

Megakaryocytes are frequently encountered normally in the femoral bone marrow of the dog. The numbers of these cells were decreased in the bone marrow of dogs in the 10 r/day group. Table 12.24 shows 14 of 16 animals in which these cells were found in decreased numbers. In none of the other groups was this finding apparent.

One instance of leukemia was encountered in the 10 r/day group. The findings in the peripheral blood in this case (Chap. 11) are compatible with a diagnosis of myelogenous leukemia; however, Engelbreth-Holm[7] points out that the most frequently observed type of leukemia in dogs is one in which visceral changes are those of lymphatic leukemia, whereas the peripheral blood shows a leucocytosis

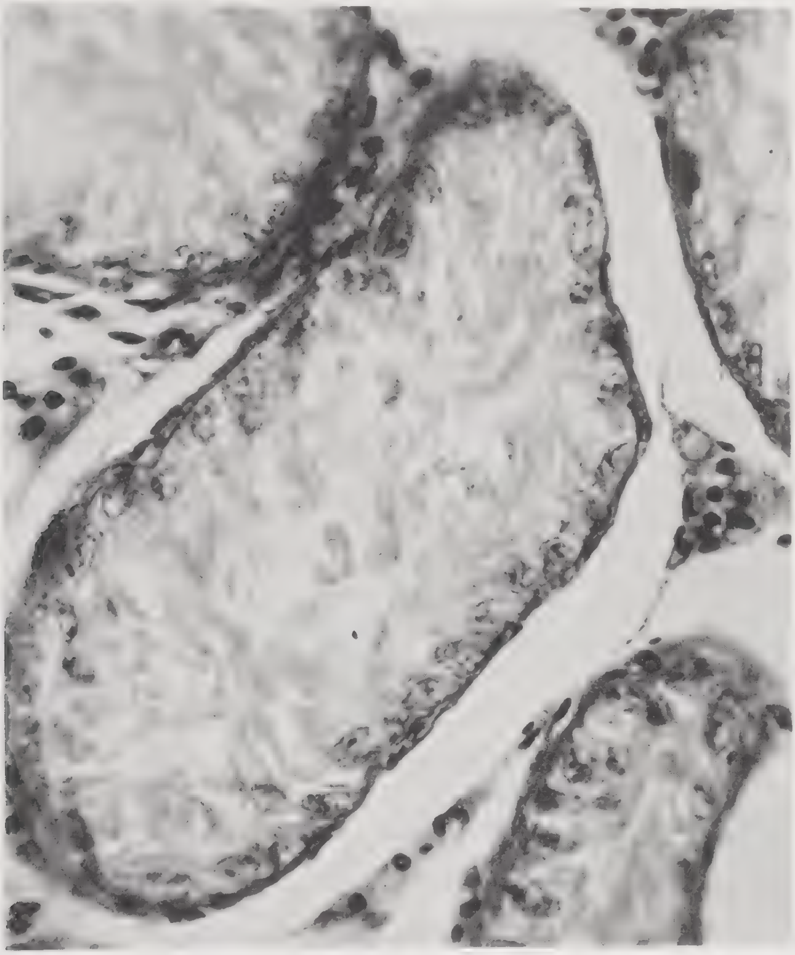

Fig. 12.17 — Similar testicular changes in dog A6 from the 10 r/day group which died after 124 treatments. 440×

in which the predominant cells are of a myeloid type. Because of the extreme immaturity of the cells in the visceral infiltrations in this case, it was not possible to identify definitely the type. There was one observation, however, which suggested that this case might fall into the above-cited category. The microscopic appearance of the cells in the bone marrow was quite different from that of the cellular infiltrations in the lymph nodes, spleen, etc. In the latter organs the cells were very large and immature, round with round vesicular nuclei and several prominent nucleoli (Fig. 12.19), whereas the marrow showed a generalized hyperplasia but only isolated islands of the very large cells.

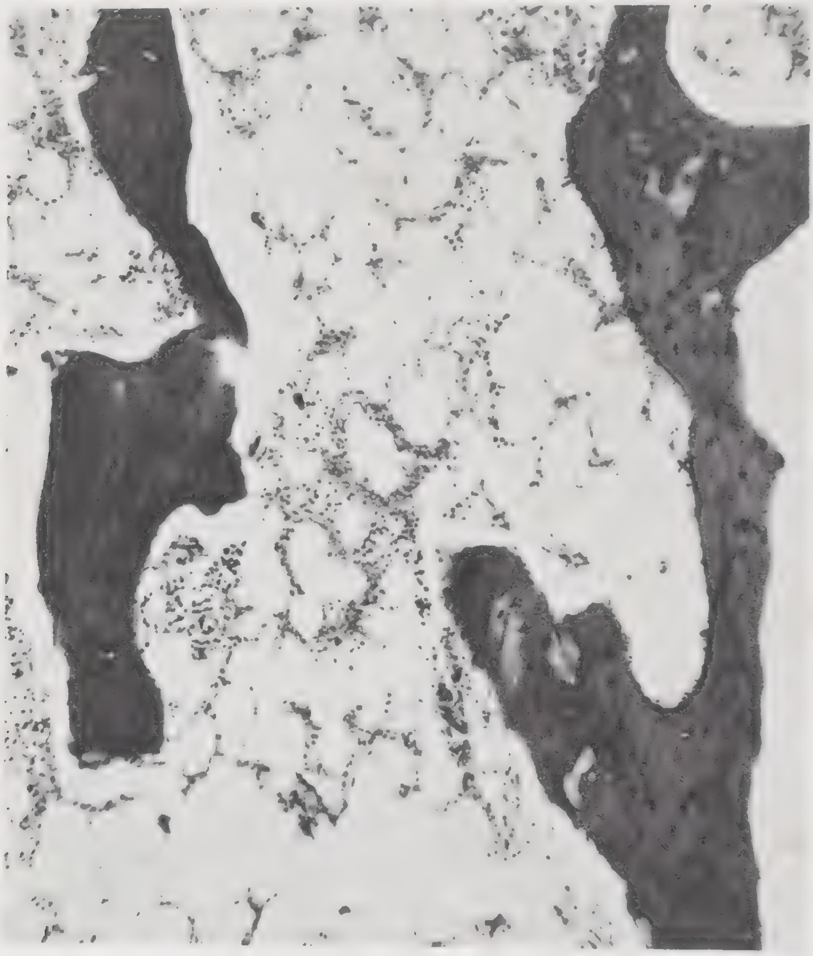

Fig. 12.18—Vertebral bone marrow from dog B9 (died after 115 treatments at 10 r per treatment), showing moderately severe hypoplasia. Large areas of the marrow cavity are occupied by fat. 100×

No other instance of tumor was encountered in the animals that died during the exposure period. Animal 1 in the 6 r/day group showed hyperplasia of the spleen and bone marrow that suggested leukemia; however, the lymph nodes did not indicate a blood dyscrasia nor was there any evidence of leukemia in the blood smears during life. It is probably safer to consider this case not as one of leukemia but as advanced hyperplasia. The thought occurred, however, that, had this animal lived sufficiently long, leukemia may have developed.

(b) Dogs Sacrificed at End of Exposure (622 Treatments). The findings in these animals are summarized in Table 12.25. Two of the animals in the 0.1 r/day group showed moderately marked splenic

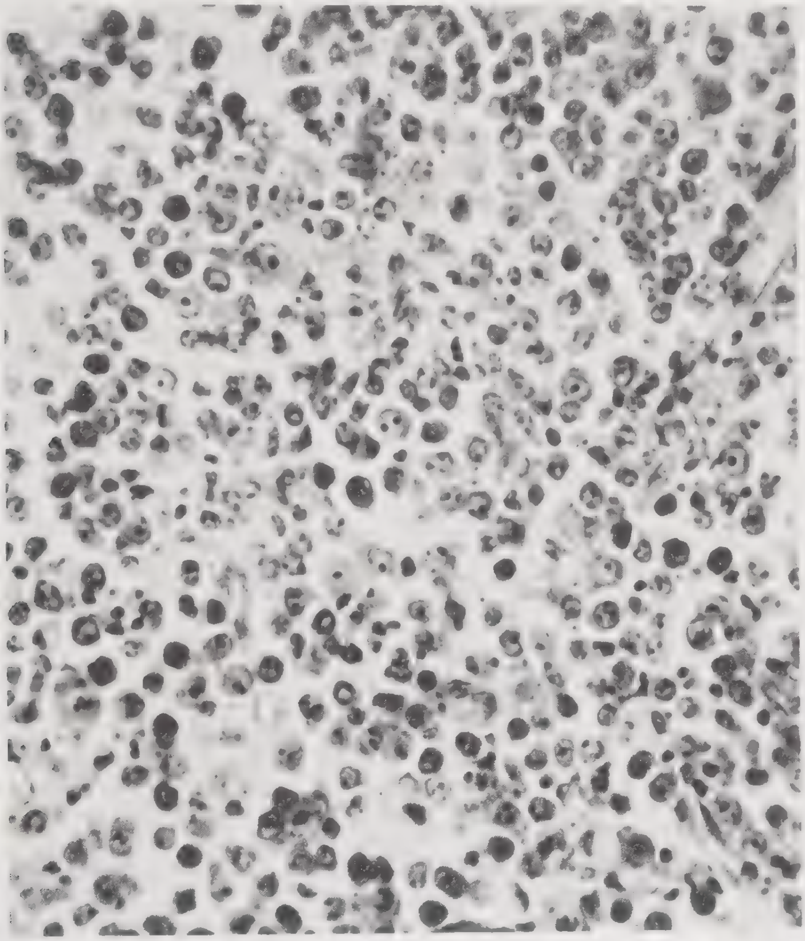

Fig. 12.19—High-magnification photomicrograph of a lymph node from dog A42 which died of leukemia after 254 treatments at 10 r per treatment. Note the large anaplastic cells which make up the cellular accumulations. 440×

hemorrhage. However, since intracardiac nembutal was used to kill the dogs, this hemorrhage may well have been caused by the method of sacrifice.

Hemorrhagic and hyperemic bone marrow was encountered in two animals in the 0.5 r/day group. This change was moderate in degree. It was often impossible in such a case to determine whether the blood in the marrow was free in the marrow cavity or confined to widely dilated sinuses. The significance of this type of change is not clear.

The number of megakaryocytes was moderately increased in the spleens of three animals in the 1 r/day group.

Testicular atrophy occurred to a marked degree in the two males examined for this lesion in the 1 r/day group. A section of testis from the third male in this group was not present for examination. Similar atrophy of moderate grade was noted in the testes of the two males in the 0.5 r/day group (Fig. 12.20).

(c) Dogs Sacrificed at End of 3 Months Rest After 622 Treatments (Table 12.26). These animals were all females. Consequently there was no testicular material for study in this group.

Splenic hemorrhage of mild to moderate grade occurred in three animals in the 0.1 r/day group, but again this lesion is discounted on the basis of the use of intracardiac nembutal for sacrifice. Bone-marrow hemorrhage was present in two animals in the 0.5 r/day group. Again this lesion is difficult to interpret because of the similarity of appearance of hemorrhage and congestion in the bone marrow.

Table 12.25—Findings in X-radiated Dogs Sacrificed After 622 Treatments*

Dose per treatment	0.1 r, 1000 kv			0.1 r, 250 kv			0.5 r, 1000 kv			0.5 r, 250 kv			1 r, 1000 kv			1 r, 250 kv		
Animal No.	3	4	5	7	9	10	5	7	10	3	6	8	1	4	7	2	6	10
Sex	F	F	M	F	F	M	F	F	M	M	F	F	F	M	F	M	F	M
Hemorrhage																		
Spleen		△			△													
Bone marrow								■				■						
Splenic megakaryocytes															+	+	+	
Hypoplasia																		
Spleen			■											□				
Bone marrow																	■	
Atrophy, testis									■	■				†		▲		▲

*The following symbols are used to denote varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

† No section examined.

Pulmonary hemorrhage was mild in three animals in the 1 r/day group.

A single instance of mammary carcinoma occurred in an animal in the 1 r/day group. This tumor was of low grade.

(d) Dogs Sacrificed After 500 Treatments. The findings in these animals are summarized in Table 12.27. Hypoplasia of the lymph nodes and bone marrow was of mild to moderate grade. This lesion was fairly consistent among the treated animals but did not appear in the controls. Hyperplasia of the lymph nodes and bone marrow was not sufficiently frequent to be of significance. Testicular atrophy was severe in all treated male animals.

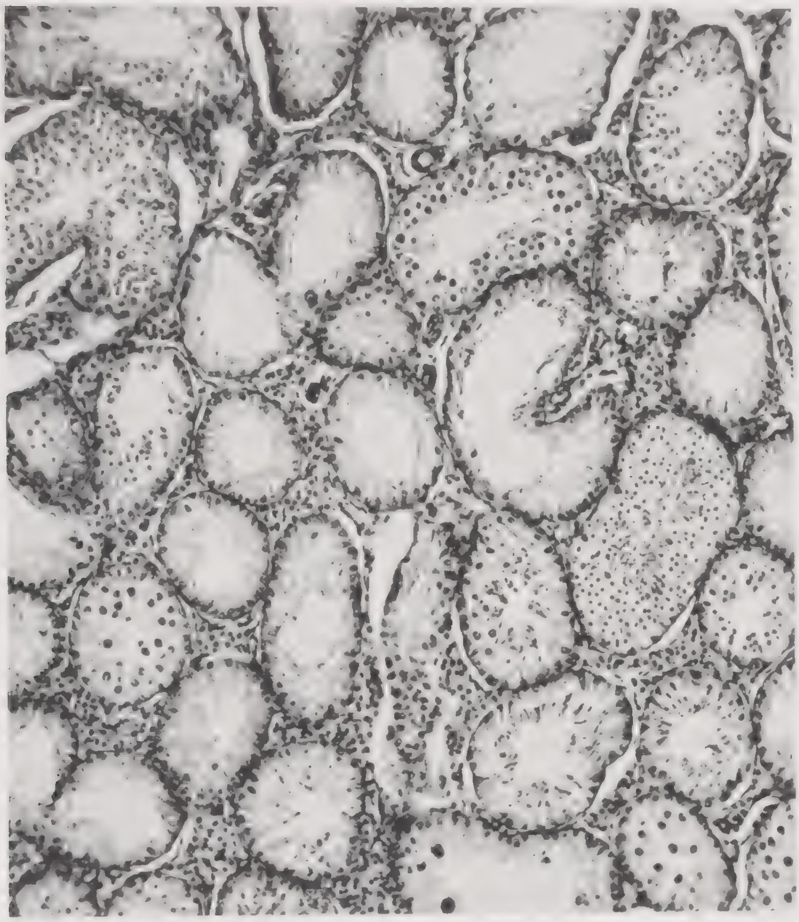

Fig. 12.20—Section of testis from dog A10 (0.5 r/day level) which was sacrificed at the end of the 2-year experiment. The seminiferous tubules show varying degrees of cellular depletion. Some tubules contain no germinal cells, some show only spermatozoa and Sertoli cells, and in others there are spermatids and spermatocytes. There are few spermatogonia in this section. 100×

Table 12.26—Findings in X-radiated Dogs Sacrificed After 622 Treatments and 3 Months Rest*

Dose per treatment	0.1 r, 1000 kv				0.1 r, 250 kv			0.5 r, 1000 kv					0.5 r, 250 kv				1 r, 1000 kv				1 r, 250 kv	
Animal No.†	2	7	9	10	2	4	8	1	3	4	6	9	1	2	4	5	2	5	8	9	5	7
Hemorrhage																						
Pulmonary																			□	□		□
Bone marrow											■					□						
Spleen			■		■	□																■
Mammary carcinoma																					+	

* The following symbols are used to indicate varying degrees of injury: +, trace; □, mild; and ■, moderate.

† All animals were female.

Table 12.27 — Findings in X-radiated Dogs Sacrificed After 500 Treatments*

Dose per treatment	10 r, 1000 kv				10 r, 250 kv		3 r, 1000 kv					3 r, 250 kv			6 r, 1000 kv		6 r, 250 kv					Control				
Animal No.	7	9	10	Freq.	6	Freq.	2	3	4	5	Freq.	3	5	Freq.	5	Freq.	1	2	3	5	Freq.	3	4	5	9	Freq.
Sex	M	M	F		F		M	M	F	F		F	F		F		F	M	M	M		F	F	F	F	
Pulmonary hemorrhage		■	□	2/3	■	1/1	□				1/4															
Splenic-follicle hypoplasia												■		1/2												
Splenic-pulp hyperplasia								■			1/4								□		1/4					
Spleen																										
Megakaryocytes								△			1/4															
Hemorrhage		■		1/3			■				1/4													■		1/4
Lymph node																										
Hypoplasia	□			1/3	□	1/1	□		■	■	3/4	□		1/2	□	1/1	■	□			2/4					
Hyperplasia								■			1/4		□	1/2					■	■	2/4					
Bone marrow																										
Hypoplasia	□	■	□	3/3	□	1/1	□		□		2/4	□		1/2				□			1/4					
Hyperplasia								△		□	2/4						■				1/4					
Testis, atrophy	▲	▲		2/2			▲	▲			2/2							▲	▲	▲	3/3					

* The following symbols denote varying degrees of injury: □, mild; ■, moderate; △, moderately severe; and ▲, severe.

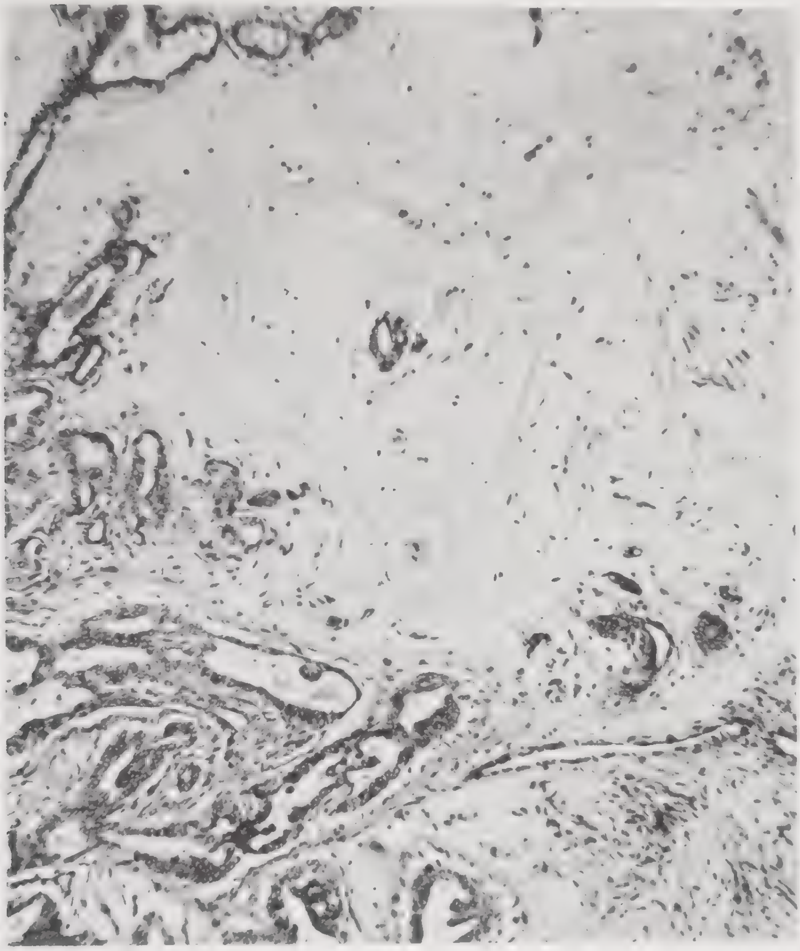

Fig 12.21—Section of a mixed tumor of the mammary gland from dog A6 in the 0.1 r/day group. This dog died after exposure was discontinued. 100×

All control animals sacrificed at this time were females; consequently no controlled testicular study was available.

The remaining lesions listed in the table were too infrequent or scattered to be significant.

(e) Postexposure Deaths. The two animals that died during the rest period were females. One showed a mixed mammary tumor that had metastasized to the regional lymph nodes and lungs (Fig. 12.21). The tumor was of the type usually seen in salivary glands.

The bone marrow in the bone sections showed mild to moderate hypoplasia. Sections of the spleen showed moderate pulp hyperplasia in one and moderate hypoplasia in the other. Lymph-node hypoplasia of mild to moderate grade was present in both animals.

There was a mild focal hemorrhage in the stomach mucosa of one of the animals.

3.4 Discussion. Histological studies of dog tissues gave somewhat more interesting results than those obtained in the study of rat and rabbit tissues. In the latter species, the only apparently significant change, aside from differences in frequency of certain neoplastic and inflammatory diseases, was in the testes.

In the dogs, not only did the testes show injury considered to be related to irradiation exposure, but certain changes in the bone marrow, intestinal tract, and lymph nodes also suggested irradiation effect.

(a) Testis. Throughout the experiment, male animals exposed to 1 r/day or more showed complete loss of germinal cells and spermatozoa. The testes of the two male animals sacrificed after 2 years of X-radiation exposure, or 622 irradiation treatments at the 0.5 r/day level, showed moderate atrophy with a decrease in the number of spermatogonia and spermatocytes in some tubular segments and complete depletion of germinal cells in others. In the same sections were many tubules showing the presence of spermatocytes and absence of spermatids and spermatozoa. Figure 12.20 illustrates the types of tubules found in the testes of these animals. Although these findings are somewhat different from those encountered in animals receiving larger daily doses of X rays and although the number of cases available for study from this group is small, the possibility that these changes were caused by the treatment must be considered since no other explanation is apparent. Furthermore, certain facts support the contention that irradiation was responsible for these changes, namely: (1) The changes were of a similar nature in the only animals in this group from which testes were examined; (2) changes of this exact type have not been observed in controls; and (3) it has been shown that the sperm counts in the dogs in this group were lower than in the controls (see Chap. 10).

In the dosage groups above 0.5 r/day in both sacrificed and dying animals, there was consistent and severe testicular atrophy. In all these male animals, all germinal cells, including spermatozoa, were absent. This finding was too consistent to be considered due to causes other than irradiation.

(b) Bone Marrow. In addition to the hypoplasia of the bone marrow, a decrease of the megakaryocytes was encountered in the 10 r/day group among the animals that died. Bone-marrow hypoplasia of moderate to marked grade appeared in these animals. A single instance of hypoplasia of mild degree occurred in the 1 r/day group. The consistency and severity of this lesion in the high-dosage group, especially in view of the known sensitivity of the bone marrow to radiation, seem sufficient indication for attributing this change to the treatment.

(c) Hemorrhagic Manifestations. Seven of sixteen animals (43.75 per cent) in the 10 r/day group that died during treatment showed bowel hemorrhage. The frequency of this lesion in the highest dose evel suggested irradiation as its cause. Hemorrhagic lymph nodes were likewise commonly observed in these dogs. Hemorrhage was observed sporadically in dogs at the lower levels. However, the frequency of such changes was not sufficiently great to allow any definite conclusion concerning their relation to the treatment.

Hypoplasia of the spleen and lymph nodes was present to some degree in most of the animals dying during the period of exposure. Except for slightly more severe lymph-node hypoplasia in certain of the 10 r/day dogs, there was no apparent relation between these changes and the exposure to X rays. In the groups of sacrificed animals, however, there was lymph-node hypoplasia only in those dogs exposed at he three highest levels. Although this change was not consistently observed in these dogs, the frequency was such as to suggest that radiation was the factor responsible.

3.5 Conclusions. 1. The consistent severe injury of the testes in male dogs used in this experiment indicates that X radiation, in doses of 1 r/day or greater, is the cause. The lack of control data does not detract from the above in the light of such consistency. Partial esticular atrophy in the two male dogs exposed at a rate of 0.5 r/day uggests an effect on the testis at this level.

2. The virtually exclusive appearance of bone-marrow hypoplasia nd decrease of megakaryocytes in the 10 r/day group and the abence of these findings in controls strongly point to X radiation as he inciting agent.

3. The presence of focal bowel hemorrhages in the animals of the 0 r/day group again suggests that X radiation has produced this hange.

4. Lymph-node hemorrhage appeared consistently in the 10 r/day nimals that died during treatment. In most instances it was severe. hese findings support the conclusion that this lesion was related to radiation at 10 r/day.

5. Splenic hypoplasia as seen in these animals did not follow a patern nor was there any marked change from the normal. Consequently o relation of this lesion to X radiation can be established. Lymph-ode hypoplasia, however, was somewhat more frequent and more arked at the three highest dosage levels.

6. There is no evidence that irradiation has caused the appearance of the two mammary tumors or the single instance of leukemia.

7. The remaining lesions listed are scattered and are mild to moderate in degree. These lesions are not considered to be related to X radiation.

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Chapter 13*

BLOOD CHEMISTRY STUDY IN DOGS EXPOSED TO CHRONIC X RADIATION

By Kathryn F. Fink

1. INTRODUCTION

The purpose of this study was to determine whether some of the biochemical components of the blood in dogs were affected by chronic X radiation at 0.1, 0.5, 1.0, and 10 r/day over a long period of time.

Serum protein was determined by the micro-Kjeldahl method. For the determination of fibrinogen the clot was formed by the method of Cullen and Van Slyke,[1] and the protein nitrogen in the clot was determined by the micro-Kjeldahl method. The nonprotein nitrogen (NPN) content of whole blood was determined by the method of Koch and McMeekin.[2] A modification of the method of Bloor, Pelkan, and Allen[3] was used to measure the cholesterol concentration in serum. Chloride analyses were made according to the procedure of Van Slyke and Sendroy.[4] Alkaline phosphatase activity of the serum was determined by the method of King and Armstrong[5] and is expressed in K.A. units. One K.A. unit is that quantity of phosphatase activity which, at pH 9.2 and 37°C, will liberate 1 mg of phenol from the disodium phenyl phosphate substrate in 30 min. The modification of King and Armstrong's procedure described by Robinson, Gutman, and Gutman[6] was used for measuring serum acid phosphatase activity, and the activity is expressed in K.A. units. One K.A. unit is that quantity of phosphatase activity which, at pH 4.9 and 37°C, will liberate 1 mg of phenol from the disodium phenyl phosphate substrate in 30 min. Duplicate analyses were made throughout.

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*Based on Report MDDC-211 (II-188-5939).

2. CONCENTRATIONS OF BLOOD COMPONENTS

The concentrations of the various components of the blood which were determined have been plotted against the number of treatments in Figs. 13.1 to 13.7. The numbers are used to represent a range of the number of treatments as indicated in Tables 13.1 and 13.2. The ranges were chosen so as to include analyses on each animal in a group. Thus each bar in the graph represents the mean value for the entire group. The groups which were exposed to 1000-kv X radiation at 0.1, 0.5, and 1.0 r/day are in the lower third of the figures, and directly above are the corresponding groups which were irradiated with 250-kv X rays. In the upper third of the figures the mean concentrations are shown, from left to right, for the controls, the group given 10 r/day of 1000-kv X radiation, and the group given 10 r/day of 250-kv X radiation. The data obtained for the five dogs in the control group have been combined and plotted over ranges which correspond in time to the ranges of the number of treatments used for the groups which were irradiated. Analyses were made each week on the control dogs and the dogs exposed to 10 r/day for about the first third of the experiment and once every 4 weeks on the groups that re-

Table 13.1 — Range of Number of Treatments at 0.1, 0.5, and 1.0 r/Day and Number of Dogs Alive

		Number of dogs alive					
	Number used to represent	1000 kv			250 kv		
Range of number of treatments	range of number of treatments	0.1 r/day	0.5 r/day	1.0 r/day	0.1 r/day	0.5 r/day	1.0 r/day
0	0	10	10	10	10	10	10
19–41	30	10	10	10	10	10	10
43–65	54	10	10	10	10	10	10
67–89	78	10	10	10	10	10	10
91–113	102	10	10	10	10	10	10
115–137	126	10	10	10	10	10	10
139–161	150	10	10	10	10	10	10
163–185	174	10	10	10	10	10	10
289–304	296	10	10	10	10	10	10
417–428	422	10	10	10	10	10	10
454–460	457	10	10	10	10	10	10
524–529	526	10	10	10	10	10	9
557–566	561	10	10	8	10	10	9
622	622	10	10	8	9	10	9

ceived 0.1, 0.5, and 1.0 r/day over the same period of time. To facilitate the comparison of results, the data for the control dogs were also calculated to correspond in time with those for the groups exposed to 0.1, 0.5, and 1.0 r/day and have been plotted in the lower right section of each figure.

Table 13.2—Range of Number of Treatments at 10 r/Day and Number of Dogs Alive

Range of number of treatments	Number used to represent range of number of treatments	Number of dogs alive	
		1000 kv	250 kv
0	0	5	5
6–11	8	5	5
12–17	14	5	5
18–23	20	5	5
24–29	26	5	5
30–35	32	5	5
36–41	39	5	5
42–47	44	5	5
48–53	50	5	5
54–59	56	5	5
60–65	62	5	5
66–71	68	5	5
72–77	74	5	5
78–83	80	5	5
84–89	86	5	5
90–95	92	5	5
96–101	98	5	5
102–107	104	5	5
108–113	110	5	4
114–119	116	5	4
120–125	122	4	4
126–131	128	4	3
132–137	134	3	1
138–143	140	3	
144–149	146	3	
150–155	152	3	
156–161	158	3	
162–167	164	3	
168–173	170	3	
174–179	176	3	
180–185	182	2	
186–191	188	2	

2.1 Serum Protein. The mean serum protein concentrations are given in Fig. 13.1. There were several general trends in serum pro-

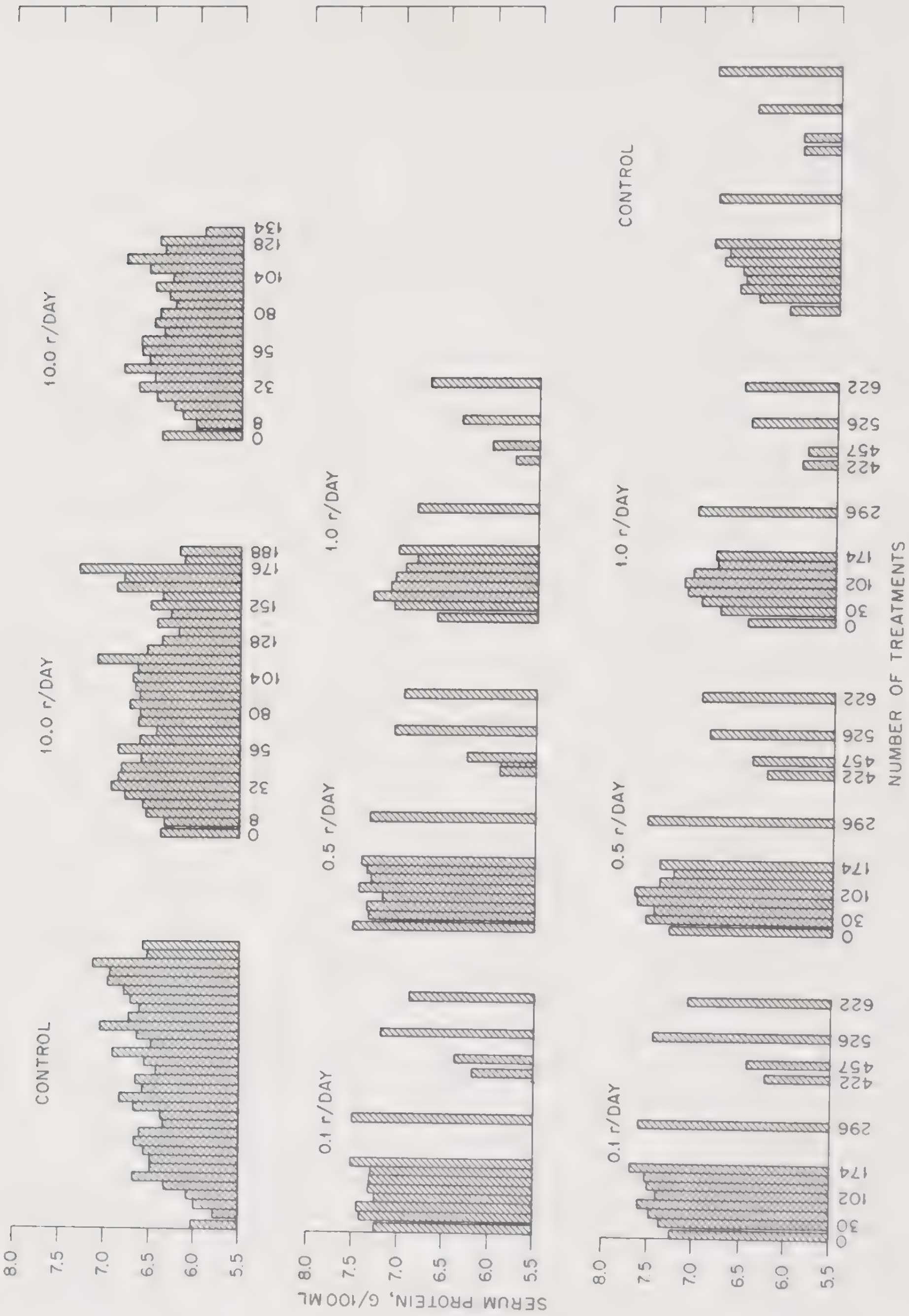

Fig. 13.1—Mean serum protein concentrations in dogs exposed to chronic X radiation.

tein levels in the control and experimental groups. Shortly after the initial control analysis was made, there was an increase in serum protein, and the higher level was maintained for about 1 year (296 treatments) after the experiment was started. The next two determinations (422 and 457 treatments) showed a pronounced drop in the concentration. A change in diet was subsequently made, and at the time of the next two analyses (526 and 622 treatments) the concentration had returned to the previous level, which would seem to indicate that the earlier drop was a result of a poor state of nutrition. The level of the control group at the end of the experiment was significantly above that at the beginning of the experiment as determined by the Fisher "t" test, whereas the corresponding levels for the irradiated groups were not significantly different. This difference between the control and treated groups is probably due to the much lower concentration of serum protein in the control animals at the time the experiment was begun rather than to the effects of irradiation in the exposed groups, since the control group exhibited the same general changes over the 2 years of observation as the experimental ones. The weekly values for the control group and the groups receiving 10 r/day show considerable intravariation but little intervariation. It should be pointed out that the values for the latter portion of the curves for the 10-r group represent fewer animals. There was no regular or abnormal change in the serum protein concentration in the dogs in the 10-r groups within 1 week before death. One of the dogs in the 1.0-r group had an abnormally low serum protein concentration of 3.6 g/100 ml after 528 exposures, 2 weeks before death.

2.2 Fibrinogen. The results on fibrinogen are shown in Fig. 13.2. The first determination of the concentration of fibrinogen in the plasma was made after the experiment had been in progress for about 3 months. With the exception of a drop found in several of the groups, including the controls, at the time of 422 treatments, the fibrinogen concentration fluctuated little. There was no significant difference in concentration among the groups at the close of the experiment. In 3 of the 10 dogs exposed to 10 r/day, increased fibrinogen levels (0.59, 0.61, and 0.62 g/100 ml) were found within 1 week before death.

2.3 Nonprotein Nitrogen. The mean NPN concentrations are given in Fig. 13.3. Both the control and the experimental groups exhibited similar trends in the concentration of NPN in the blood throughout the 2 years of observation. The levels were increased above those of the initial analysis shortly after the radiation was begun and were considerably elevated at a time corresponding to 174 treatments. At the times corresponding to 296 and 422 treatments, the levels had

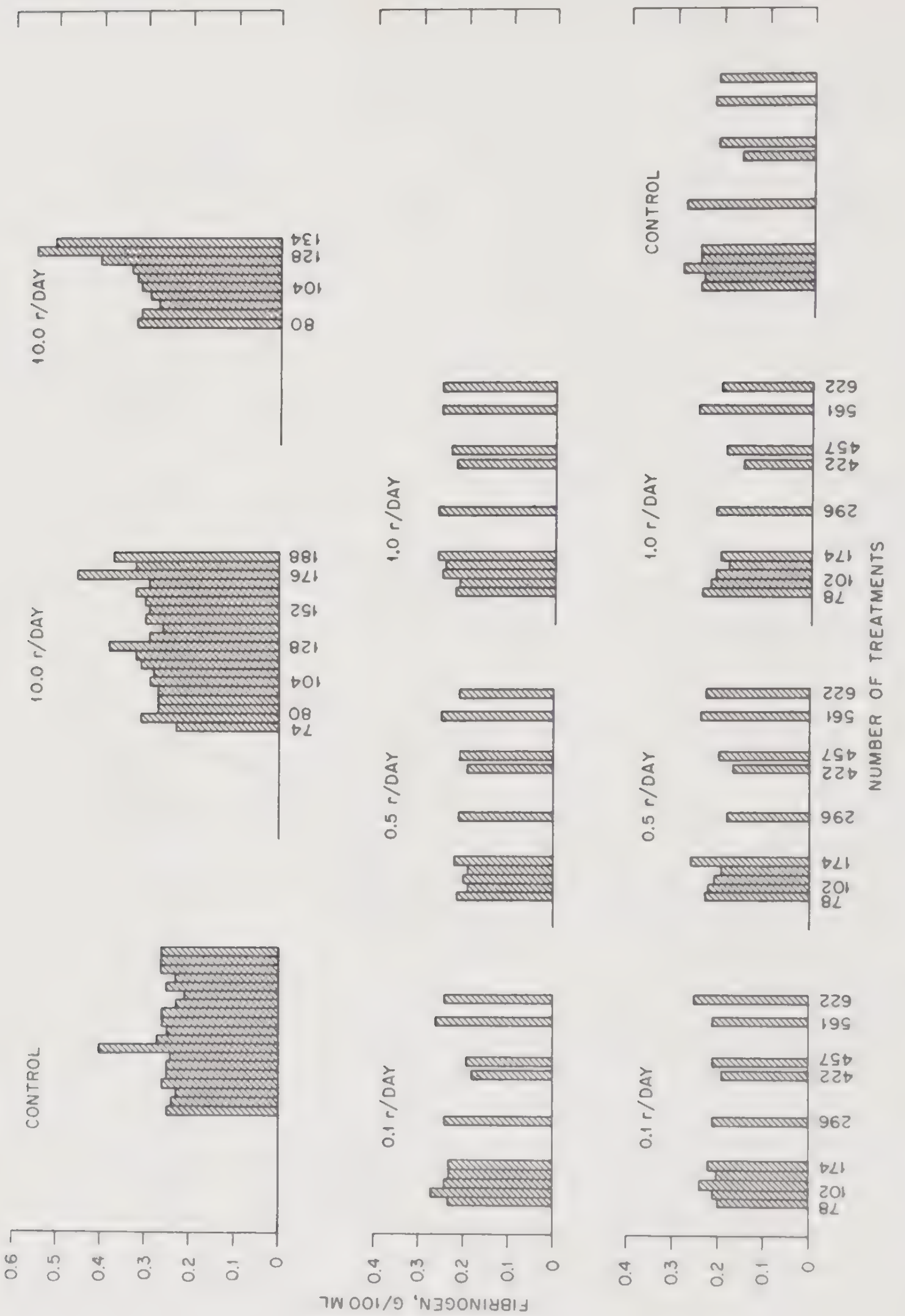

Fig. 13.2—Mean fibrinogen concentrations in dogs exposed to chronic X radiation.

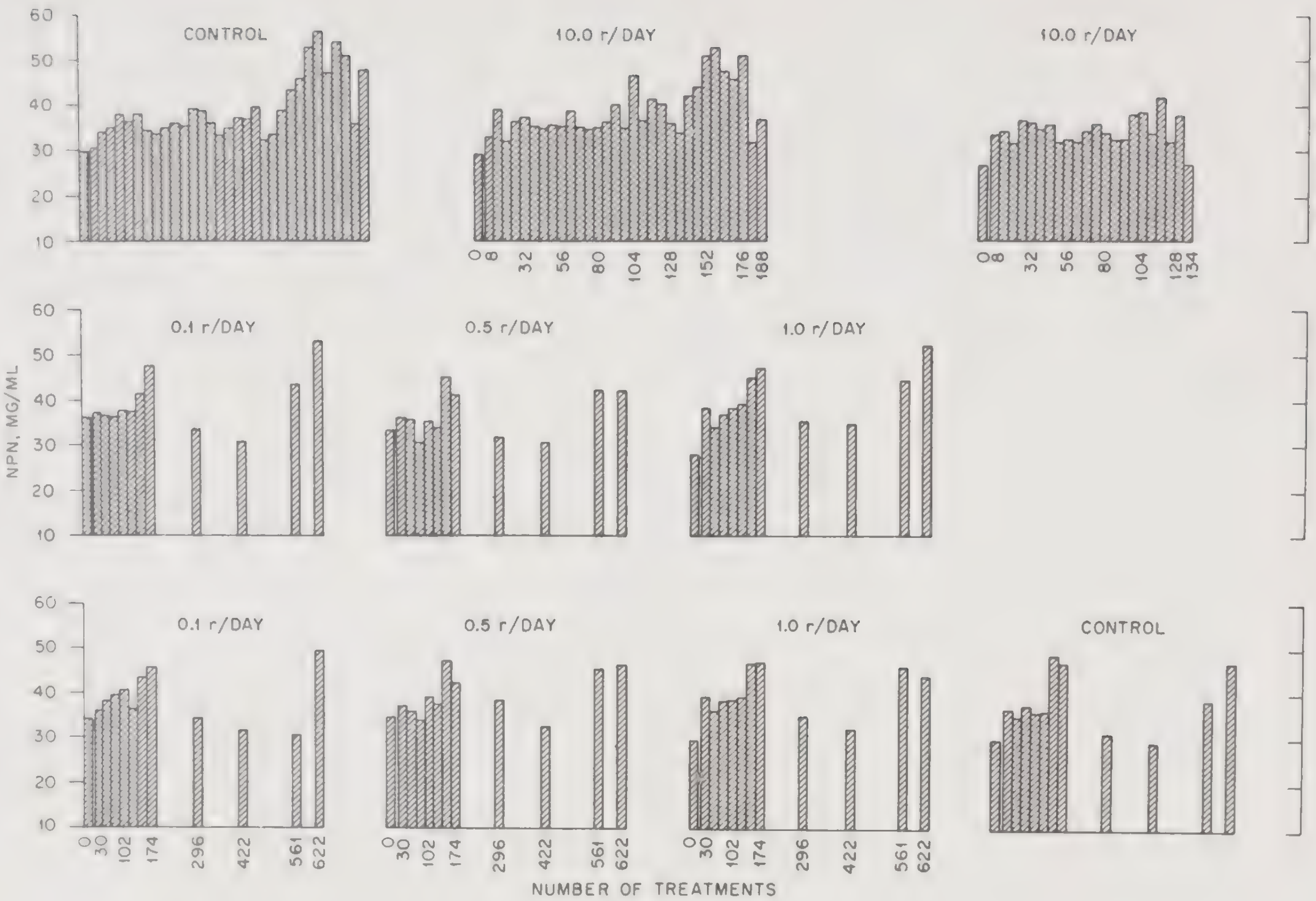

Fig. 13.3 — Mean NPN concentrations in dogs exposed to chronic X radiation.

dropped to such an extent that they were comparable to the initial control concentrations. At the conclusion of the experiment the levels were significantly above the original control levels. No consistent changes were found in the concentration of NPN in the animals that died.

2.4 Serum Cholesterol. Serum cholesterol values are plotted in Fig. 13.4. The cholesterol concentration was below the initial level at a time corresponding to 422 treatments in all the groups of dogs, including the controls. At the conclusion of the experiment the levels were remarkably comparable to the initial analysis for all the groups except the one exposed to 250-kv X radiation at 1.0 r/day. The increase in this group was not statistically significant according to the Fisher t test. The concentration in the samples taken was affected little in the dogs receiving 10 r/day even within 1 week previous to death.

2.5 Serum Chloride. The mean serum chloride concentrations are shown in Fig. 13.5. The first analyses for this constituent were made after the experiment had been in progress for about 6 weeks. There were no consistent variations among the groups. The most pronounced change was at the time of 296 treatments, when the levels were the highest observed for the control group, the three groups treated with 1000-kv X radiation, and the group given 1 r/day of 250-kv X radiation. There was little difference in the mean values among the various groups at the close of the experiment, and there were no abnormal changes in the dogs that died.

2.6 Serum Acid Phosphatase. The mean serum acid phosphatase concentrations are given in Fig. 13.6. The concentration of serum acid phosphatase was elevated somewhat in the experimental groups after 296 treatments. With the exception of that rise, the variation of the means within and among groups was remarkably small. No unusual findings were present within a week previous to death.

2.7 Serum Alkaline Phosphatase. The mean concentrations of serum alkaline phosphatase are shown in Fig. 13.7. The activity of the enzyme was not determined until the experiment was in progress for some time. In general, the experimental groups had slightly lower activity than the control groups, but trends among the groups were similar. Two of the ten dogs in the 10-r groups had abnormally high alkaline phosphatase concentrations (11.0 and 31.6 K.A./100 ml) within 1 week before they died, whereas the concentrations previous to this time were normal. It is of interest that these two dogs also had high fibrinogen levels (0.61 and 0.62 g/100 ml) at the same time. The abnormal concentrations of alkaline phosphatase found in

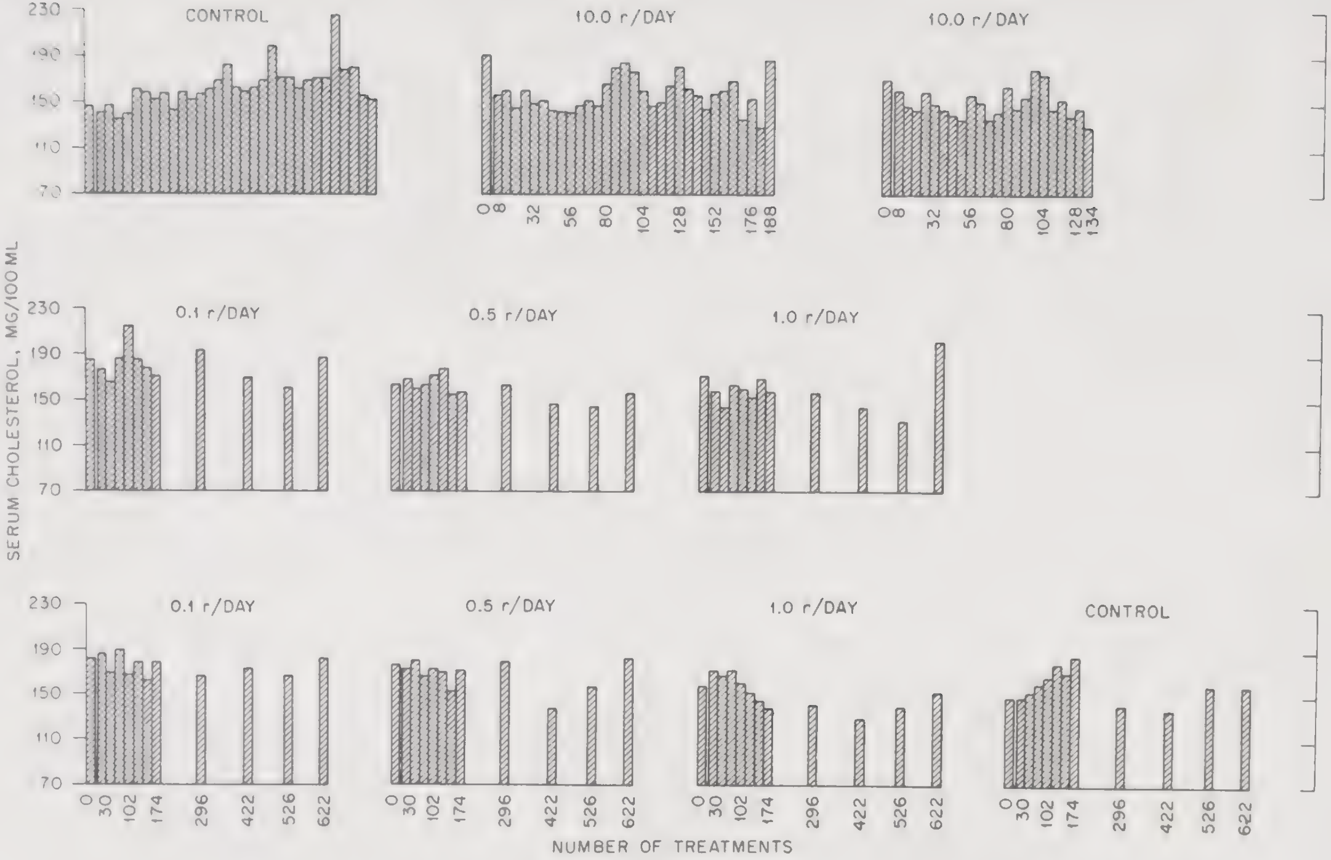

Fig. 13.4 — Mean serum cholesterol concentrations in dogs exposed to chronic X radiation.

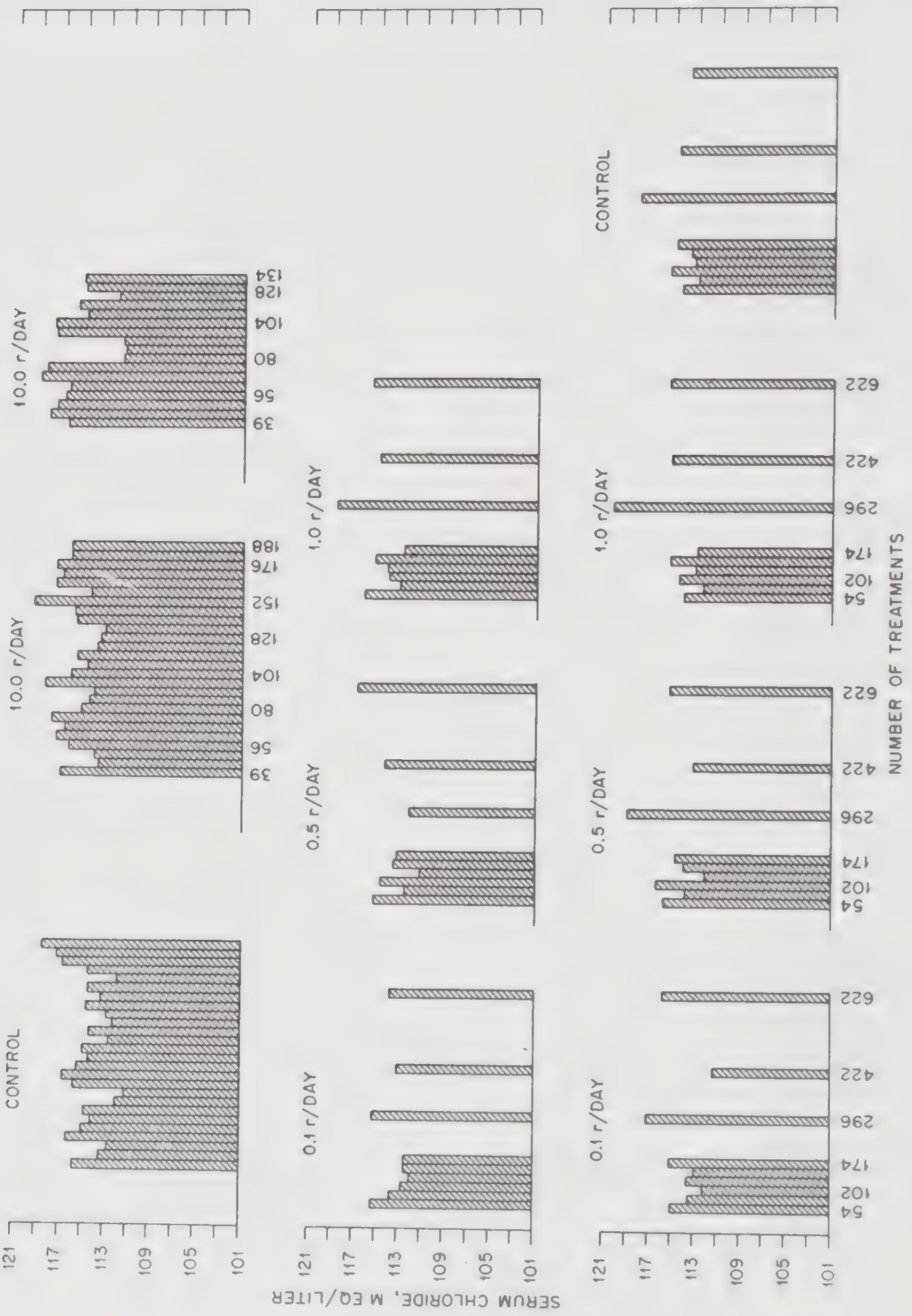

Fig. 13.5—Mean serum chloride concentrations in dogs exposed to chronic X radiation.

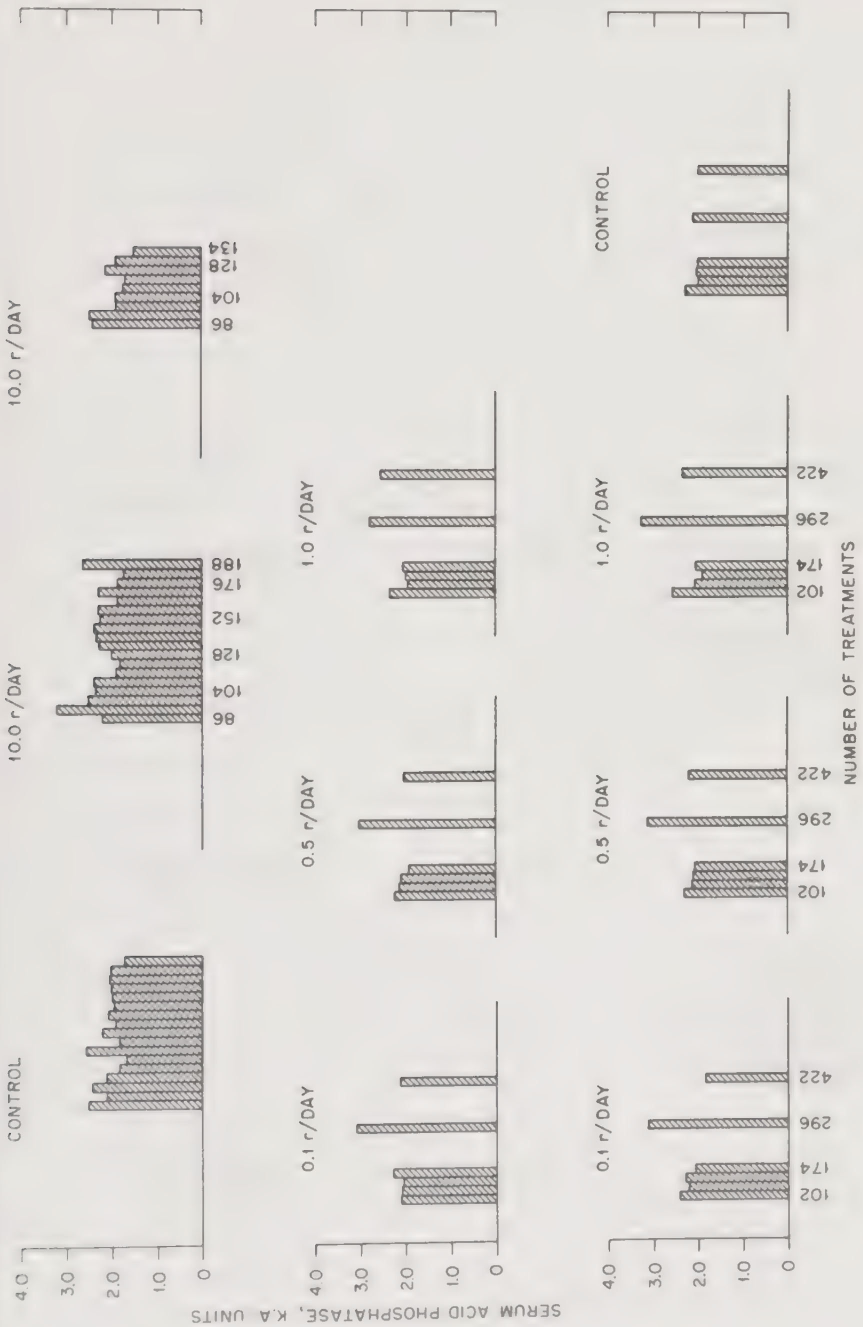

Fig. 13.6—Mean serum acid phosphatase concentrations in dogs exposed to chronic X radiation.

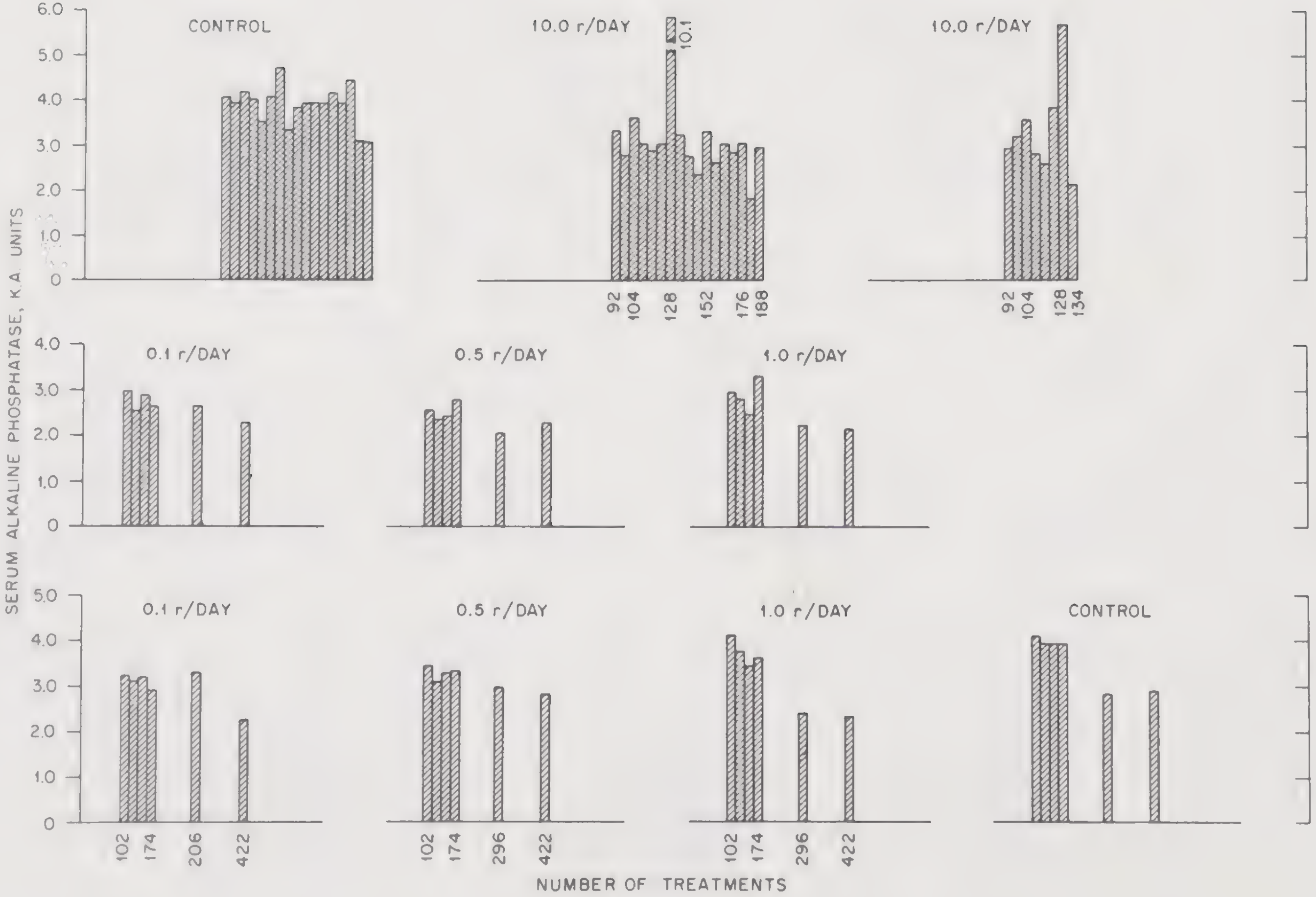

Fig. 13.7—Mean serum alkaline phosphatase concentrations in dogs exposed to chronic X radiation.

the serum of these two dogs are responsible for the high mean levels in the 10-r groups at 128 treatments.

3. SUMMARY

The concentrations of serum protein, plasma fibrinogen, whole-blood NPN, serum cholesterol, serum chloride, and serum acid and alkaline phosphatases were not significantly altered in dogs subjected to chronic X radiation at 0.1, 0.5, 1.0, and 10 r/day, 6 days a week. All the dogs in the 10-r groups and four dogs in the remaining groups succumbed before the experiment was terminated at the time of 622 exposures. There were no consistent changes even in the dogs which died as a result of the radiation. The only obvious changes observed in the dogs given 10 r/day which died were high fibrinogen concentrations in three of the dogs, two of which had high alkaline phosphatase levels. These changes did not develop until about a week before the animals died; so, even if they had occurred in all the dogs which died, they would have been of little practical value as an index to radiation damage.

ACKNOWLEDGMENTS

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Chapter 14*

EFFECT OF SHORT-WAVELENGTH RADIATIONS ON HUMAN FINGER RIDGE DETAIL

By Roger A. Harvey

The occurrence of dryness and atrophy in the skin of the hands following prolonged exposure to X and radium rays has been known and admitted from the days of the pioneer radiologists. The prevalence or degree of such changes in hands of professional groups has never been determined accurately because this was considered important only to a few people and as inevitable consequences of certain professions. An opportunity to investigate the possible types and degree of changes which could occur arose when detection of minimal radiation effects became of interest to the Manhattan Project.

The fingerprint area was selected for investigation because it was possible that some of the industrial and research groups connected with the Project might receive high intensities of radiations to the fingers if adequate protective measures were not provided and enforced. It is admitted that the skin in this area is relatively resistant to radiations,[1] but, if changes occur secondary to radiations, they might show up as altered morphology of ridges of the fingerprints.

A search of available fingerprint literature failed to reveal any specific information applicable to this problem. Cummins and Midlo[2] grouped changes resulting from leprosy and X rays together by stating that, when the damage is slight or superficial, the original characteristics of the skin may be restored and that deeper invasion of the disease process permanently effaces dermatoglyphics in the region involved. Most of the literature deals with identification problems, and, apparently, radiations have not caused sufficient alteration in fingerprints to prevent identification, nor have changes been encountered often enough in the past to warrant special investigation.

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* Based on Report MDDC-216 (II-188-5941).

At the start the problem appeared to be divided into three sections: (1) an evaluation of methods of recording fingerprints, (2) a study of possible changes produced in fingerprints following exposure of fingers to radiations, and (3) application of methods and findings to and for the safety of large groups of people employed on or in conjunction with the Manhattan Project.

1. SURVEY OF METHODS

Various kinds of inks and papers, impression materials, and casting and modeling compounds were evaluated. The final selection of the red dental compound manufactured by the Kerr Mfg. Co., Detroit, Mich., was influenced by the detail recorded, ease of recording fingerprints, low cost, permanence, and absence of interfering material. The selection of this particular product does not constitute an endorsement of it for its intended dental use over other similar products because in this work it was softened by different methods and to lesser extents than is intended for dental purposes.

This impression compound is hard and brittle at room temperature and softens at 132°F. Many methods can be used to heat it, but running hot water or a hot-water bath is best suited for isolated examinations, and infrared lamp heating is best when large groups of people are to be examined at one time. Water near the boiling point is undesirable because it alters the color and causes a crackled surface later which interferes with interpretation. Dry heating methods often liquefy the surface to such an extent that sticking of the compound to the fingers interferes with making a good impression. This difficulty is easily overcome if the fingers are gently wiped with a cloth moistened with mineral oil before the impressions are made. It is best to place the compound on stiff cardboard in order to prevent curling or buckling of the compound when it softens. As soon as the edges of the compound feel as soft as putty, sufficient heat has been applied, and the compound and cardboard should be placed on a firm flat surface. The impressions are obtained by placing the finger parallel with the surface of the compound and starting contact with the finger turned slightly to one side of the fingerprint area. The finger is then pressed into the surface of the compound, slowly turned over the volar surface to the opposite edge of the finger, and gently lifted from contact with the compound. The best impressions are 1 to 2 mm in total depth into the compound. Any attempt to pick up the block of compound before it hardens results in rippling of the surface which interferes with subsequent study.

2. COLLECTION OF DATA

During the period of evaluation of different methods of recording finger impressions, over 200 different people were studied. Impressions from several radiologists were included in this group, and their fingers showed changes which were not duplicated by any of the others, even from the same age group. It seemed expedient to follow up this clue for information first and to leave a study of animal footpads before and after various intensities and fractionations of short-wavelength radiations for ultimate investigation.

Table 14.1 — Collection of Data

	Radiologists	Orthopedists	Dentists
Number of sets of compound sent out	2300	749	550
Number of satisfactory tests returned	1059	186	503
Number of unsatisfactory tests returned on first trial	14	1	
Number of sets returned because addressee was overseas, ill, or deceased	133	36	
Number of addressees who specifically refused to contribute to the survey	25	12	
Number of dentists in New York State			11,800
Number of dentists in New York State who admitted exposure of hands to X rays at some time during their career			550

Explanatory letters, questionnaires, and impression materials were sent to all the registered radiologists in this country, subsequently to orthopedic surgeons because many of them receive radiations to their hands during fluoroscopic reduction of fractures or while holding cassettes, to dentists in New York State who admitted holding films in mouths of patients during X-ray exposures, to radiologists in Canada, and to a miscellaneous group of radiation physicists, radiation research workers, and patients whose hands had been treated with X or radium rays. Simultaneously, control impressions were collected from local hospital personnel, patients, and preemployment examinations of people hired for the Manhattan Project (see Table 14.1).

Returns from the survey of radiologists were allowed to accumulate until 300 sets of impressions were available for review. They were examined with a magnifying lens several times in an attempt to devise a system of classification for grading the changes observed.

Two systems were developed. The first was simply to separate the impressions into normal or abnormal groups. The second system was one of degree of change observed, and the following categories were set up for convenience of classification:

1. No change
2. Early flattening of ridges; localized, patchy, or generalized
3. Localized atrophy of minimal degree
4. Localized atrophy of moderate degree
5. Localized atrophy of advanced degree
6. Generalized atrophy of minimal degree
7. Generalized atrophy of moderate degree
8. Generalized atrophy of advanced degree
9. Atrophic fissures
10. Proliferative changes

Table 14.2—Radiologists in Each Exposure Interval Exhibiting Fingerprint Change*

Exposure, years	Frequency	Number exhibiting no change	Number exhibiting change	Per cent exhibiting change
43–47	10	2	8	80
38–42	28	1	27	96
33–37	31	2	29	94
28–32	70	2	68	97
23–27	191	25	166	87
18–22	169	26	143	85
13–17	165	53	112	68
8–12	165	81	84	51
2–7	204	141	63	31
Total	1033	333	700	68

*Median age, 46.98 years; median years in radiology, 16.85.

It is admitted that many of these changes can occur from causes other than short-wavelength radiations, but their development, course, and relative incidence in exposed and unexposed groups are the important differentiating points.

In Table 14.2 are summarized the findings in impressions submitted by the radiologists. For the purpose of this study the material has been interpreted simply as showing change or no change. Table 14.3 is a more detailed summary of the findings in impressions from radiologists. The numerical headings under "Number of changes" refer to the classification of changes as outlined above; number 1 signifies no change in ridge detail, number 2 early abnormalities, etc., to number 10 which shows the number of individuals exhibiting proliferative changes, but only on the volar surface of the finger. In

Table 14.3 — Changes Observed in Finger Impressions of Radiologists in Each Exposure Interval

Exposure, years	Number of cases	Number of changes									
		1	2	3	4	5	6	7	8	9	10
43–47	10	2	4	3	2		5	1		2	3
38–42	28	1	15	8	4		7	3		6	8
33–37	31	2	12	8	3		14	2		2	10
28–32	70	2	47	23	3		16	8	1	8	19
23–27	191	25	127	58	12		37	6		20	36
18–22	169	26	113	57	9		24	5		15	24
13–17	165	53	101	32	3		6			3	4
8–12	165	81	79	23	1		1			2	
2–7	204	141	61	13	2						
Total	1033	333	559	225	39		110	25	1	58	104

Table 14.4 — Radiologists Showing Changes in Finger Impressions After Various Intervals in Practice

Exposure, years	Per cent showing changes		
	X ray only	X ray plus radium	Radium only
43–47	50	87.5	
38–42	90	100	
33–37	94.7	91.6	
28–32	96.9	100	100
23–27	85	87.6	100
18–22	81.5	85.5	90
13–17	71.4	66	66
8–12	41	54.7	44
2–7	24	31.7	33

Table 14.4 the data from Tables 14.2 and 14.3 are divided into groups according to practice with X rays only, X rays and radium, and radium only. Further analysis related to the use of radium reveals that atrophic fissures and proliferative changes occur 3 times more frequently in the groups that handled radium than in the group that used X rays only. Tables 14.5 and 14.6 were derived from the data sub-

mitted by orthopedic surgeons, and Tables 14.7 and 14.8 were derived from the data submitted by dentists.

In Fig. 14.1, derived from the data in Tables 14.2, 14.5, and 14.7, the percentage of radiologists, orthopedists, and dentists showing changes in finger impressions is plotted, respectively, against years in practice in radiology and orthopedics and, in the case of dentists, against years of admitted film-holding rather than years in practice. The variation in slopes of the different lines is a contradiction of the observed effect being based on age alone.

Table 14.5—Orthopedists in Each Exposure Interval Exhibiting Fingerprint Change*

Exposure, years	Frequency	Number exhibiting no change	Number exhibiting change	Per cent exhibiting change
38–44	5	1	4	80
33–37	5	1	4	80
28–32	8		8	100
23–27	21	4	17	81
18–22	29	10	19	66
13–17	47	20	27	57
8–12	51	34	17	33
3–7	20	13	7	35
Total	186	83	103	55

*Median age, 44.31 years; median years in orthopedics, 14.50.

Table 14.6—Changes Observed in Finger Impressions of Orthopedists in Each Exposure Interval

Exposure, years	Number of cases	Number of changes									
		1	2	3	4	5	6	7	8	9	10
43–47	2		1	1	1		1			1	
38–42	3	1	2								
33–37	5	1	4	2						1	
28–32	8		8	4	3					1	3
23–27	21	4	17	3	2						1
18–22	29	10	19	2			1			1	
13–17	47	20	27	3	1					1	
8–12	51	34	17	5							2
2–7	20	13	7	1							
Total	186	83	102	21	7		2			5	6

Breakdown of the data from radiologists in Fig. 14.1 into groups of approximately 500 radiologists each for statistical evaluation reveals some interesting differences (see Fig. 14.2). Group A includes returns from the first 488 radiologists submitting data and is made up mostly of names beginning with letters in the first half of the alphabet. Group B includes returns from the next 550 radiologists to answer the request and are mostly from names beginning with letters

Table 14.7 — Dentists in Each Exposure Interval Exhibiting Fingerprint Change*

Exposure, years	Frequency	Number exhibiting no change	Number exhibiting change	Per cent exhibiting change
38–43	2		2	100
33–37	3	1	2	67
28–32	19	8	11	58
23–27	50	21	29	58
18–22	88	44	44	50
13–17	94	59	35	37
8–12	98	45	53	54
3–7	67	48	19	28
0–2	82	78	4	5
Total	503	304	199	40

*Median age, 46.13 years.

Table 14.8 — Changes Observed in Finger Impressions of Dentists in Each Exposure Interval

Exposure, years	Number of cases	Number of changes									
		1	2	3	4	5	6	7	8	9	10
43–47	1		1	1	1					1	
38–42	1		1	1							
33–37	3	1	2								
28–32	19	8	11	6	4					1	2
23–27	50	21	28	6	2					2	3
18–22	88	44	44	9	3					3	1
13–17	94	59	34	6	4					1	2
8–12	98	45	53	15	1		1			3	5
0–7	149	126	23	8	2					1	2
Total	503	304	197	52	17		1			12	15

in the last half of the alphabet. Group C is an overlapping group beginning with return number 250 and including consecutive returns to number 767. Figure 14.2 shows the relation of the regression lines for each of these groups to each other.

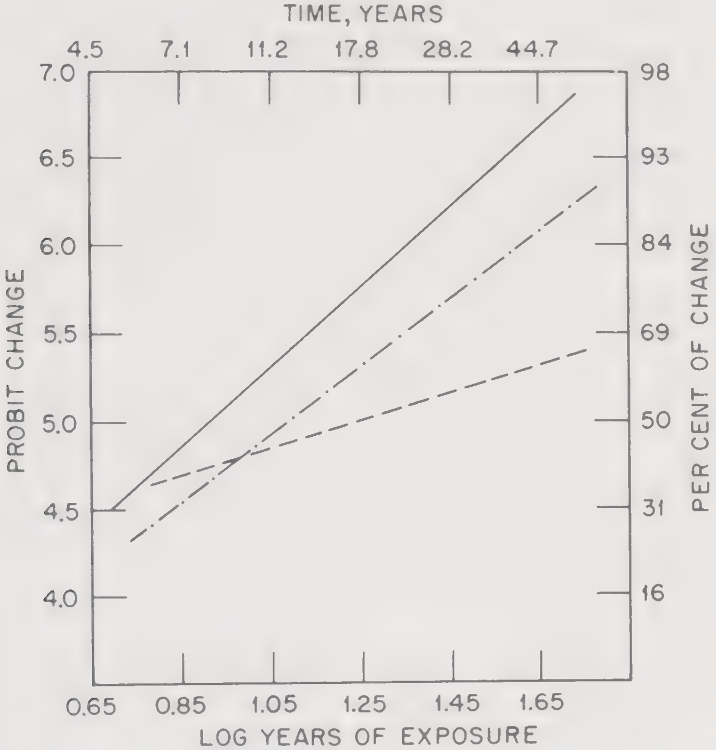

Fig. 14.1—Regression lines for predicting fingerprint change with years of exposure. ——, radiologists. – – –, dentists. ·–·–, orthopedists.

An investigation into possible explanations for the differences in the regression lines for groups A, B, and C was carried out. A good possibility was that more caution was exercised in interpreting impressions as experience was gained. Therefore the entire collection of impressions was reinterpreted without reference to the findings of the first interpretation and, finally and independently, by a second trained interpreter. The results of these laborious procedures yielded no explanation for the differences in the regression lines but did yield a strikingly high degree of constancy of interpretation of the same impressions. For example, there was only 1 case out of 1038 in which a second interpretation yielded a reading of no change which had been considered positive on the first reading. In the first reading there were a total of 1121 changes detected, and in the independent rereading there were 26 instances in which the degree of change differed by one place in the classification system. Analysis of information given on the questionnaires, which were returned with the impressions, failed to reveal any differences between the groups which could be based on age of the individual, number of years each had spent in radiology, number of hours of fluoroscopy per week, or method of protection during fluoroscopy. However, Group A did have an average of 11.46 radium cases per month per man using radium against 14.09 radium cases per month per man using radium in Group B. Although this difference is not statistically significant, it is the only difference discovered between the groups which might possibly account for the differences in the regression lines in Fig. 14.2.

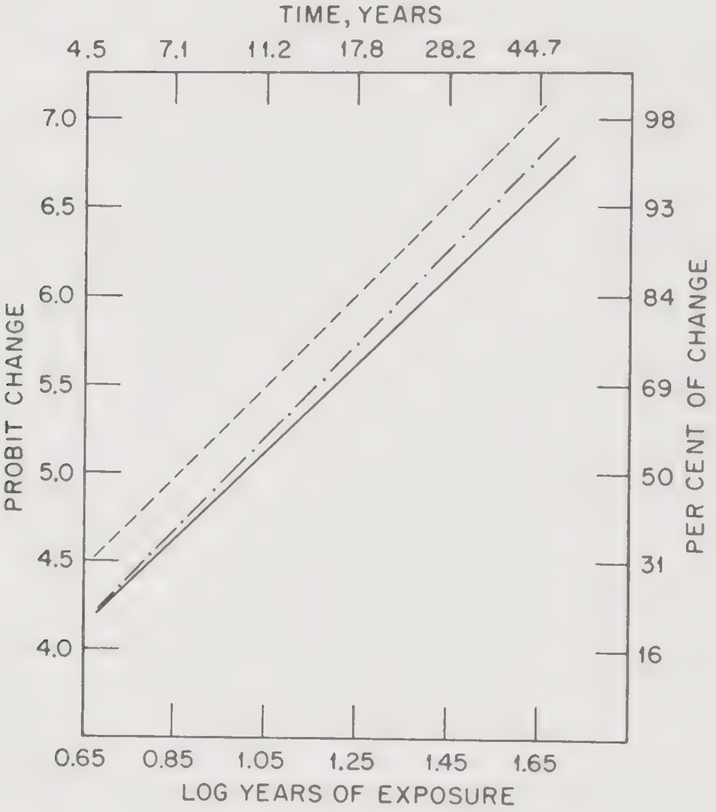

Fig. 14.2—Regression lines for predicting fingerprint change with years of exposure for radiologists. ——, group A. ---, group B. -·-·-, group C.

3. CONTROLS

The control material consisted of impressions from 1850 people. Of these, 200 were obtained during preliminary study of methods of recording finger ridge detail, 90 from unexposed radiologists, orthopedists, and dentists, and the remainder from hospital personnel, patients, and preemployment examinations. The positive findings totaled 1.8 per cent.

In some of the preemployment tests of the Manhattan Project, there were several examples of questionable or suspicious changes in ridge details. Such interpretation was meant to imply recognition of abnormality but uncertainty as to classification. These questionable or suspicious findings averaged less than 5 per cent.

4. INDUSTRIAL TESTS

A total of 4243 impressions were received from industrial groups associated with the Manhattan Project. Most of the examinations were precautionary in nature, but positive findings were observed in 20 per cent of the impressions submitted from radiation-exposed groups. These changes were mild in most cases, and, at the time of writing, no case of extensive or progressive atrophy had been detected.

Breakdown of the industrial data into two groups, one consisting of impressions from the largest contributor and the other consisting of impressions from all other industrial sources in the country, showed 18 per cent definite abnormalities in the first exposed group and 22 per cent in the second exposed group.

5. DISCUSSION

The specificity of radiation effects in finger impressions is questionable. On the basis of material analyzed in these surveys, this type of examination seems to be a relative test. There are two ways of enhancing its specificity, the first being a series of impressions on any case under question, and the second being control impressions from people of the same age, sex, and occupational background as the one being investigated.

There are many forms of trauma which can cause changes in finger ridge contour but only a few which can cause confusion with radiation effects in finger ridge details. Abrasives and caustics cause the most trouble in single examinations, but their effects are usually of much shorter duration than radiation effects. Tailors and shoemakers frequently show permanent changes from needle punctures, infection, and resulting scarring. Reynaud's disease tends to duplicate radiation effects when the disease has progressed to gross atrophy of terminal phalanges. Prolonged intake of arsenic results in dermatitis and arsenical keratoses, all of which can be most confusing. Exfoliative dermatitis, fungus infections, and blisters from heat have not caused appreciable difficulty in this work. Whenever peeling occurs in these cases, the new ridge detail is already present in the underlying layer.

At the conclusion of the surveys of professional groups, it was felt that any change produced in ridges by radiation was of a permanent character. This conclusion seemed justified in view of the persistence of changes in ridges for years after the last known exposure and only slight improvement after prolonged vacations in other cases. Since that time, periodic examinations have been made on several young radiologists who showed minimal changes and patients who had been treated with X rays for verrucae of the volar surface of the fingers or toes. On the basis of these latter studies, complete regeneration of ridges can occur although it is a very slow process. Whether ridges completely repair and stay normal or repair and subsequently weaken or never regenerate appears to be entirely dependent on the amount of exposure.

The quantity of radiation required to give changes in ridges cannot be estimated from these studies. From observation of industrial tests, it is known that a few workers who have reported for duty with a clinical erythema of the hands and fingers have ultimately shown changes in ridges. There is a delay of 4 to 8 weeks between appearance of erythema and detectable alteration in ridge detail. Clinically, flattening of ridges has been demonstrated around verrucae treated with X rays in doses of 800 r and up measured in air.

The quality of radiation required to produce changes is apparently in the beta and gamma ranges. Repeated impressions from 90 workers who were possibly exposed to only alpha rays have shown no changes. Impressions from 10 workers possibly exposed to soft beta rays also have failed to show changes. The remainder of the possible exposures have been in the shorter wavelength beta and gamma range, and the positive findings have been predominantly confined to these groups. The site of action of radiations in producing these changes in finger ridges has not been clearly demonstrated. The irregular granular character of ridges during regeneration would suggest that some of the cells in the basal layer of the papillae are more sensitive to radiations than others. As a result, newly formed cells heap up over the most viable basal-layer cells in contrast to the intervening stunned or slower regenerating cells which are unable to produce a grossly detectable collection of cells above them.

The effectiveness of protective devices or methods used by professional groups cannot be evaluated from this work, although the need for protection is certainly obvious. Most doctors have changed their methods of protection several times during their practice, and it is therefore impossible to ascribe damage now detectable to any one period of practice. However, the material does show that the less the protection, the greater the chances are of changes being observed in ridge detail. There were four radiologists who were able to correlate their damage with split seams in protective gloves after damage in the finger impressions was reported and two who knew that they had changes from the same cause before they submitted their impressions to us. A few radiologists, who are right-handed, routinely apply radium with their left hand to lessen the danger to the right hand which they use most in fluoroscopy. The left hands showed the greatest changes in these individuals. Some dentists showed minimal changes in their fingers and reported "roentgen ulcers" or "roentgen cancer" of the wrists. Apparently the patient's cheek and teeth and film holders held back radiation from the fingers, whereas the bare wrist received full exposure from a poorly coned X-ray machine. Without exception the dentists who worked in mental institutions showed the greatest changes in their finger ridges. Often their patients are incapable of holding films themselves during examination and will not tolerate the devices designed to hold films. Many of the orthopedic surgeons admitted the possibility of heavy exposure of their hands to X rays during their period of training, on a busy service, or during apprenticeship. Most of them stressed that they either have given up use of the fluoroscope themselves or else use it cautiously. The survey of orthopedic surgeons missed a large group of surgeons who do orthopedic work although they are not certified. This latter group is rumored to have many outstanding examples of severe X-ray damage, but there is no way of finding a significant number of them easily. A few of them have heard of this study, have approached us, and in turn have submitted impressions which rival the most severe changes observed in the combined X-ray and radium groups.

Surprisingly few of the radiologists and orthopedic surgeons had any idea of the amount of X rays being emitted from their fluoroscopes. The few who had measured the output reported extremes of 7 to 54 r/min in air at the tabletop.

Figures 14.3 to 14.8 are photographs of finger impressions showing various degrees of radiation damage. Figure 14.9 is a photograph of

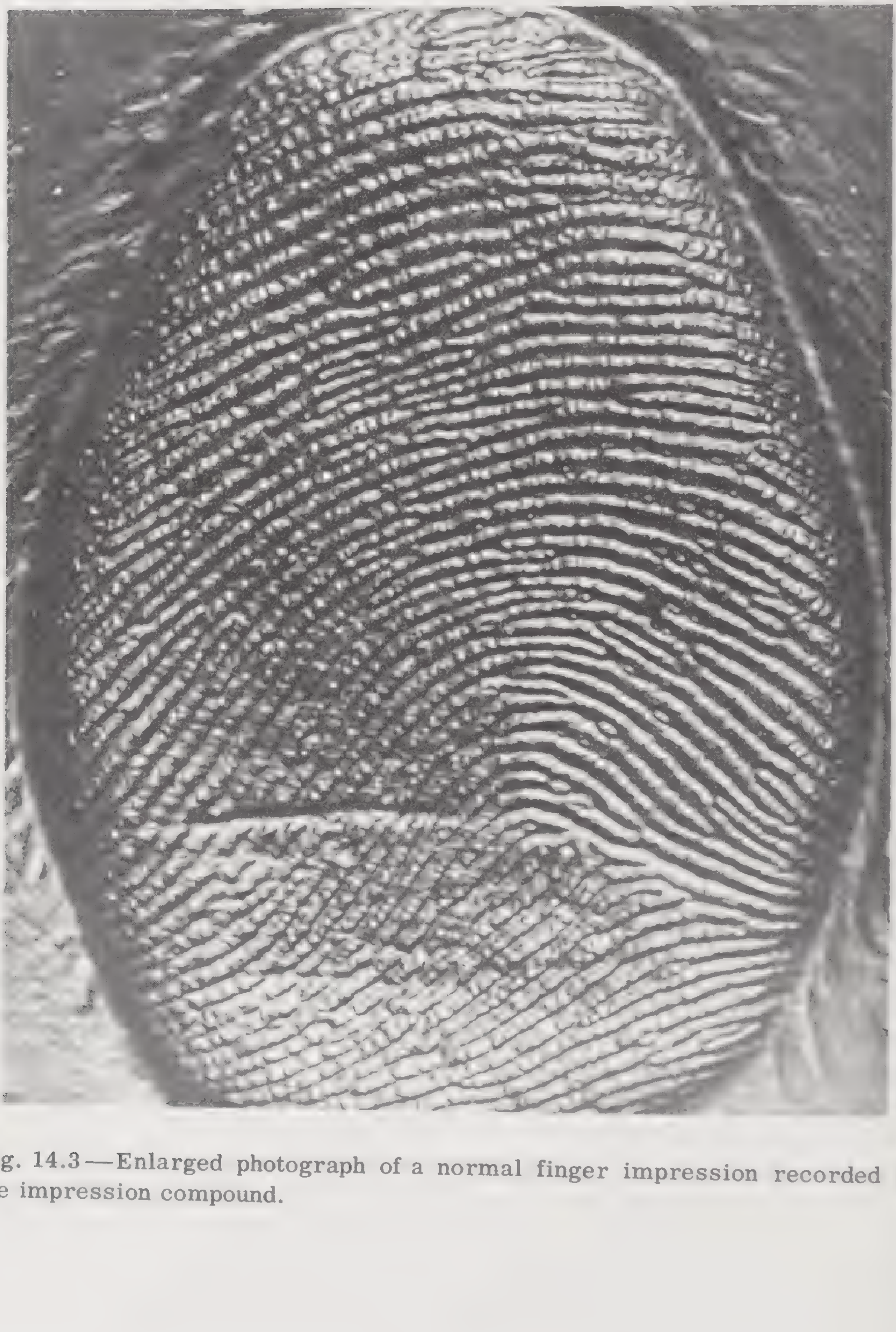

Fig. 14.3—Enlarged photograph of a normal finger impression recorded in the impression compound.

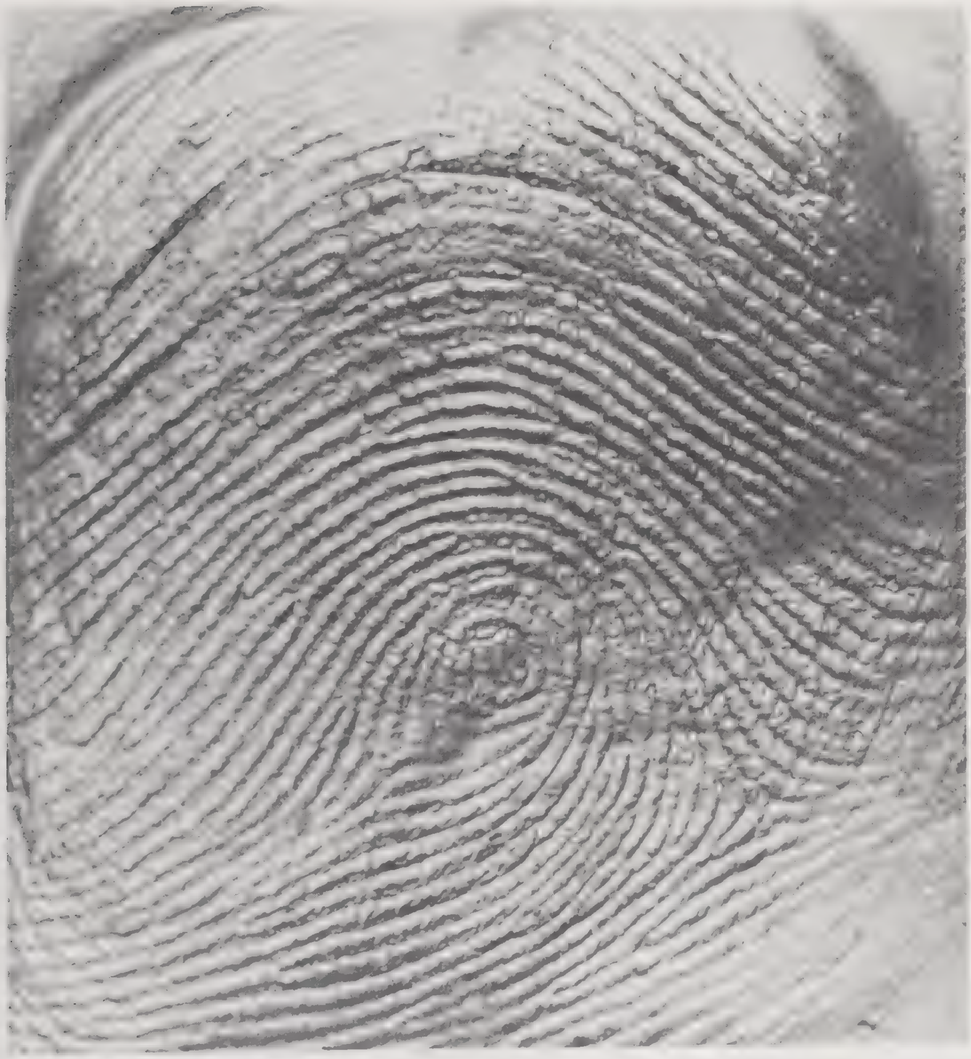

Fig. 14.4—An impression submitted by a radiologist who had practiced 5 years with X rays and radium. Early flattening and localized atrophy of the ridges are demonstrated.

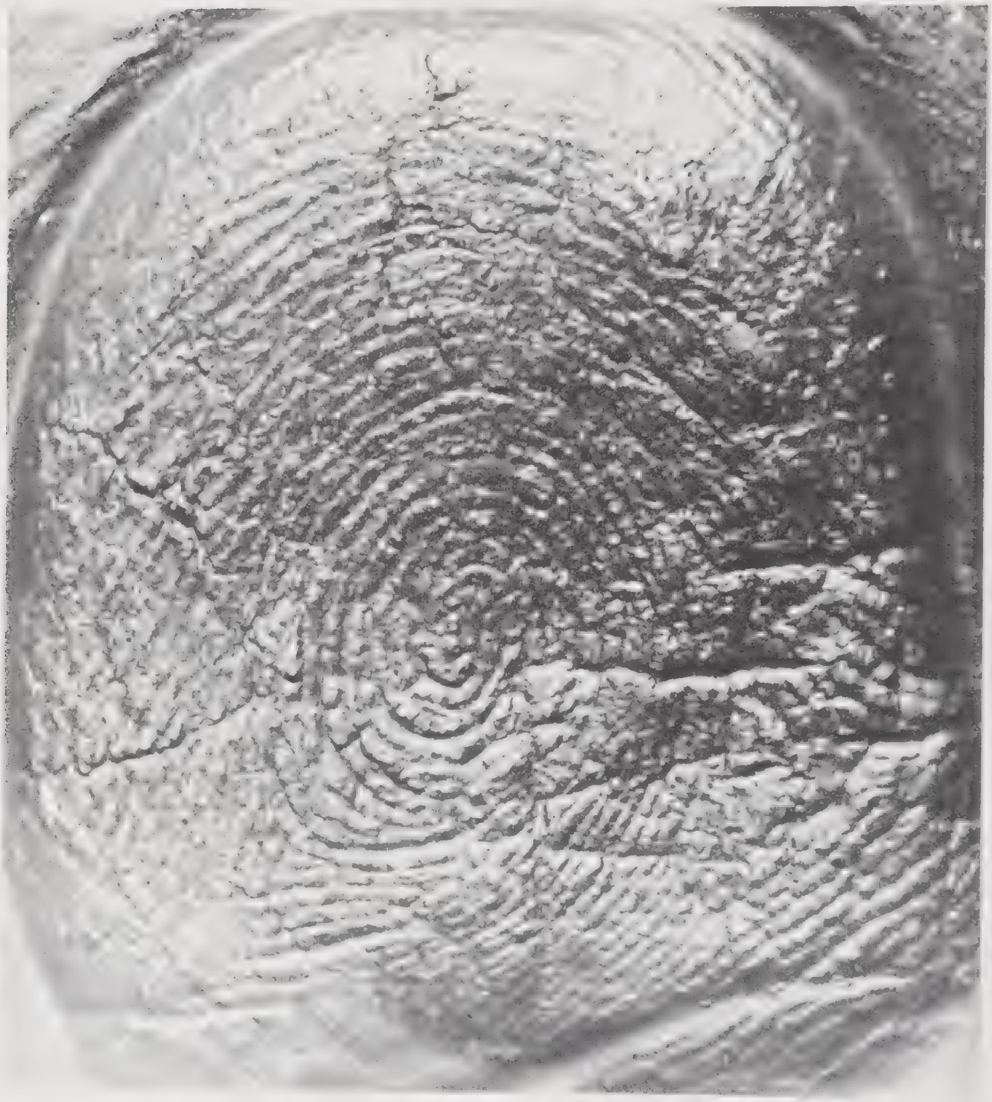

Fig. 14.5—An impression submitted by a radiologist who had practiced 15 years with X rays. Generalized atrophy and atrophic fissures are present.

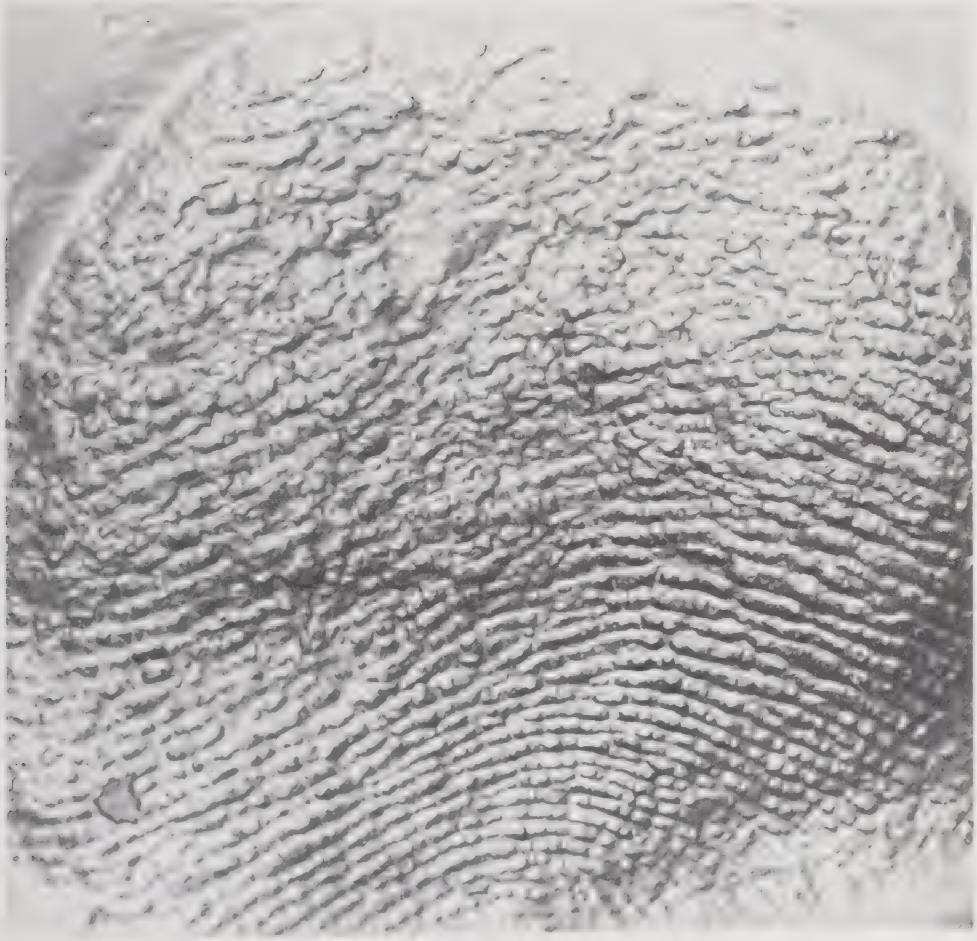

Fig. 14.6—An impression submitted by an orthopedic surgeon who had used a fluoroscope for 20 years in his practice. Flattening of the ridges, localized atrophy, and proliferative changes are present.

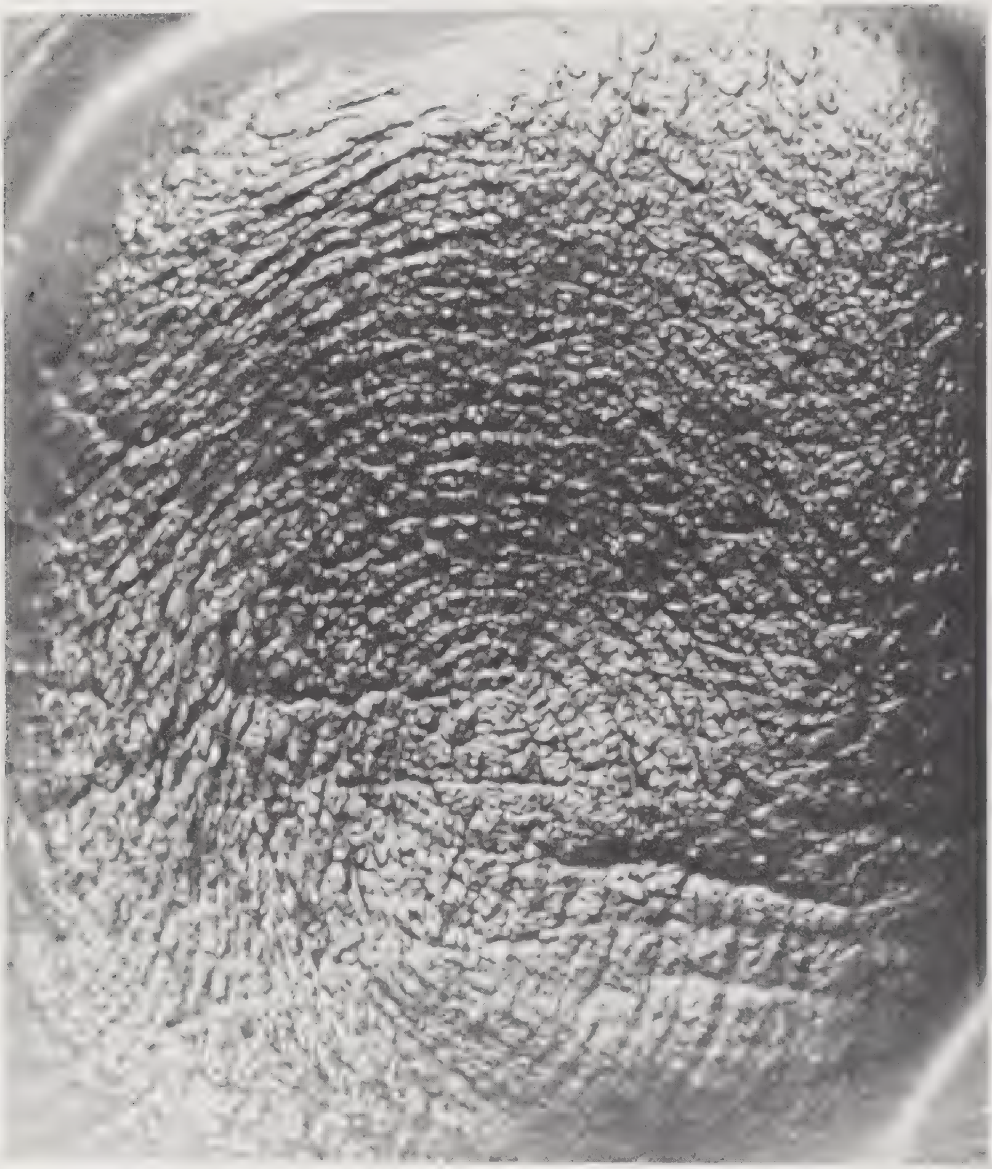

Fig. 14.7—An impression submitted by a dentist who had held films in patients' mouths during X-ray examination intermittently for 20 years. Early flattening and localized atrophy are present.

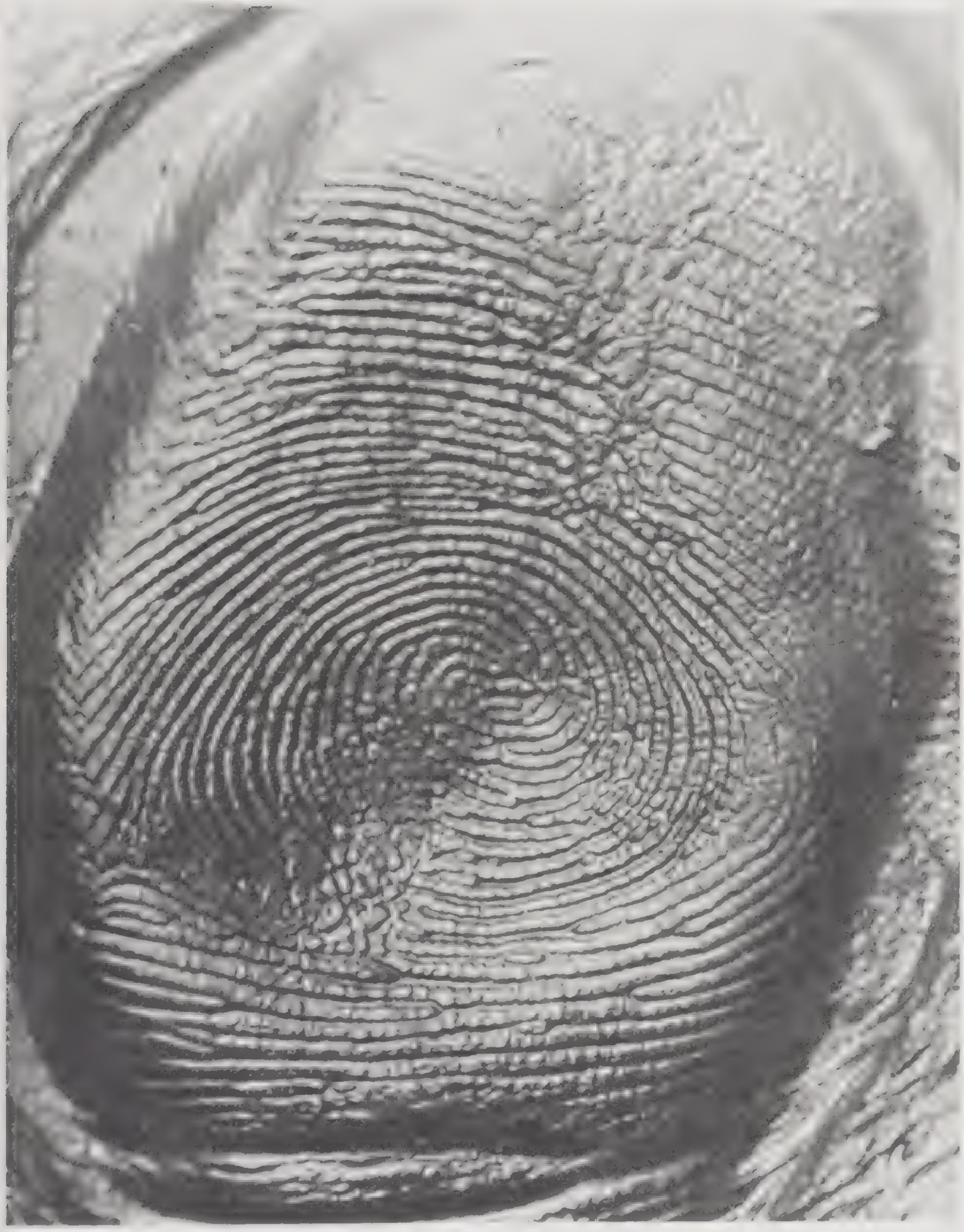

Fig. 14.8—An impression submitted by a 24-year-old worker who was industrially exposed to radiations by finger or glove contamination. The flattening of the ridges is obvious along the tip and side of the finger.

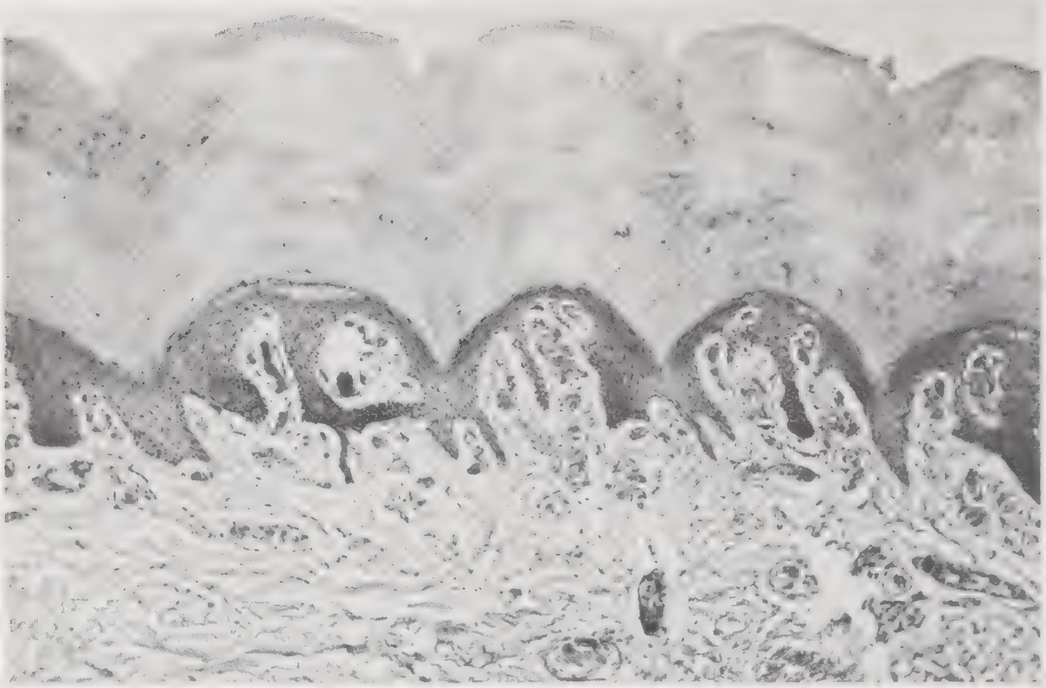

Fig. 14.9—A section of the index fingerprint area from an adult male without known exposure to X or radium rays. 40×

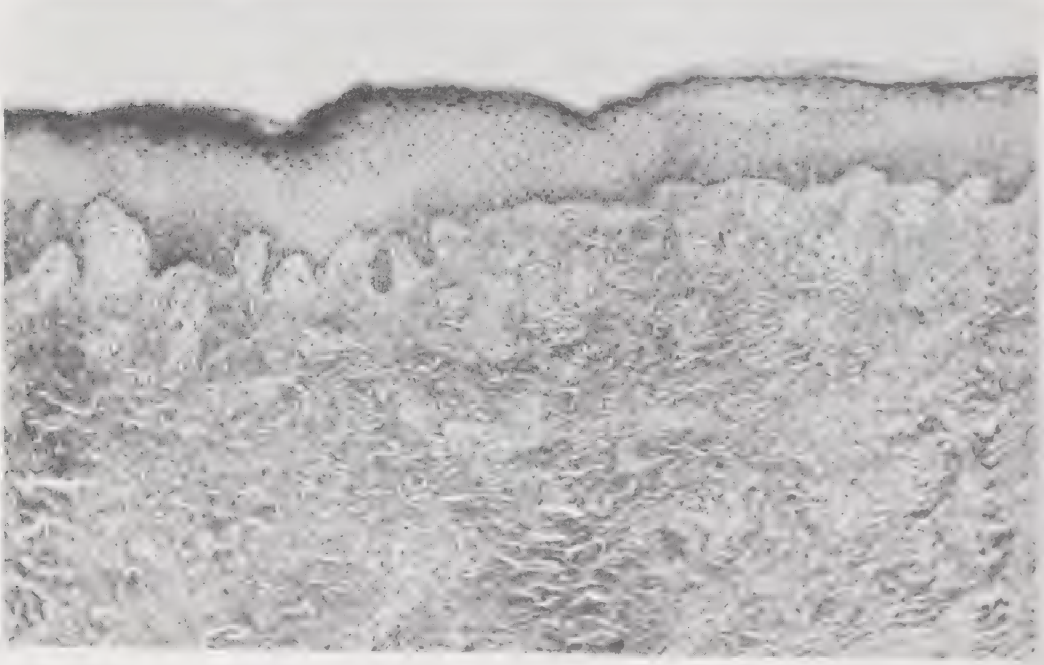

Fig. 14.10—A section of the index fingerprint area from an adult male with known excessive exposure to X rays 1 year previously. 40×

a section of the index fingerprint area from an adult male without known exposure to X or radium rays, whereas Fig. 14.10 is a section of the index fingerprint area from an adult male with known excessive exposure to X rays 1 year previously. The main difference between Figs. 14.9 and 14.10 can be summarized as follows:

Figure 14.9	Figure 14.10
Thick uniform contoured outer layer; stratum corneum	Thin flattened outer layer
Thin arched dermal papillae	Thick flattened granular papillae
Prominent blood supply in each papilla	Inconspicuous blood supply to papillary area
Loose reticular area beneath papillae containing blood vessels and sweat glands	Firm, dense, fibrotic layer, infiltrated with inflammatory cells; absence of glands; diminished blood-vessel content

6. SUMMARY

An attempt was made to develop a method of detecting short-wavelength radiation injuries in finger ridge detail for the protection of the personnel of the Manhattan Project.

Impression compound was found to be best suited for reproduction of finger ridge detail for this test.

Basic surveys on professional groups to determine the possible types and degree of injuries which can be produced by these radiations reveal that 68 per cent of 1033 radiologists, 55 per cent of 186 orthopedists, and 40 per cent of 503 dentists cooperating in preliminary studies showed changes in finger ridge details which were classified into groups according to severity of findings.

Impressions from 1850 control people yielded 1.8 per cent showing changes similar to the radiation-exposed groups and as high as 5 per cent of preemployment tests showing questionable or suspicious findings.

Application of this test to 4243 possibly exposed employees on the Manhattan Project yielded 20 per cent positive findings.

The specificity of this test for detecting radiation injuries is based, in addition to the previous figures, on the following:

1. Normal findings in radiologists, orthopedists, and dentists who have never had exposure to radiation.

2. Right-handed radiologists who have a habit of applying radium with the left hand showed greatest changes in the left hand.

3. The incidence of atropic fissures and keratoses in the fingers of radiologists who used radium was 3 times that for radiologists who had never used radium.

4. Areas of changes in ridge detail coincided with areas of split seams in the protective glove.

5. The most pronounced changes in dentists were noted in the group who work in mental institutions and have to hold most dental films during exposure to X rays.

6. In the industrial group, 90 workers who were in the position to be exposed to alpha rays showed no changes.

7. Changes in the industrial group were most commonly noted after possible exposure to beta and gamma radiation.

The specificity of findings can be enhanced by repeated impressions on any one case under investigation and by impressions from controls of the same age, sex, and background as the one under question.

Confusion is most likely to arise following use of abrasives or caustics, but regeneration following these agents is more certain and usually more rapid than after radiation injury. Reynaud's disease and long-standing arsenical dermatitis of the hands caused confusion in interpreting the results.

7. CONCLUSIONS

Atrophy of ridges of the fingerprint area has been detected in 68 per cent of the radiologists, 55 per cent of the orthopedists who use a fluoroscope, 40 per cent of the dentists who hold films during exposure to X rays, and 1.8 per cent of the controls.

The findings in professional groups were subclinical in a high percentage of cases, and they should not cause alarm if the standards of protection are met.

Application of findings from the professional groups to industrial groups capable of possible radiation exposure in connection with the Manhattan Project has been made. Although 20 per cent of the material submitted from selected groups showed changes in ridge detail, the damage was detected in an early state, and increased protection, reevaluation of employee methods, and rotation of personnel could be effected for employee safety.

REFERENCES

1. G. M. MacKee, "X-rays and Radium in the Treatment of Diseases of the Skin," Lea & Febiger, Philadelphia, 1938.
2. H. Cummins and C. Midlo, "Fingerprints, Palms and Soles," The Blakiston Co., Philadelphia, 1943.

Chapter 15*

INFLUENCE OF CHRONIC IRRADIATION WITH GAMMA RAYS AT LOW DOSAGES ON MUTATION RATE IN DROSOPHILA MELANOGASTER

By Ernst Caspari and Curt Stern

1. INTRODUCTION

The influence of radiation of short wavelength on the mutation rate in Drosophila has been measured repeatedly since the pioneer work of Muller.[1] As a general rule it was found that the mutation rate is directly proportional to the dose of radiation, as expressed in roentgens. This linear proportionality between radiation dose and mutation rate applies to all dosages of X rays and gamma rays tested, up to the time of writing, except for the highest dosages, in which a "saturation effect" comes into play. At the low end of the curve, Spencer and Stern (Chap. 7) found the proportionality maintained down to a dose of 25 r.

It was furthermore found that at high and medium dosages the mutation rate was independent of the intensity, i.e., of the time over which the application of a certain number of roentgens was spread. This was established by Patterson[2] and Oliver[3] and others for X rays and by Hanson and Heys[4,5] and Raychaudhuri[6] for gamma rays. Timoféeff-Ressovsky and Zimmer[7] have calculated that, in all experiments, a dose of about 3600 r would result in a mutation rate of 10 per cent.

The experiments reported in this chapter have been undertaken to study the question of whether or not the rule that the mutation rate is independent of the time of irradiation also holds for low dosages.

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*Based on Report MDDC-1200 (II-188-7027; M-1966), August 1946, which has appeared in the published literature [Genetics, 33: 75-95 (1948)].

Although it is well established that, at high and medium dosages, the same number of roentgens induces the same number of mutations regardless of whether it is applied at once or spread over a considerable time, this question has not been investigated at very low dosages.

2. MATERIALS AND METHODS

The methods were chosen in such a way as to be comparable to those used by Spencer and Stern in their experiments on acute irradiation at low dosages. There were, however, a certain number of differences.

The irradiation was done with gamma rays, as opposed to Spencer and Stern's X rays. A needle containing 10 mg of radium was used as the source of irradiation. The flies to be irradiated were kept in shell vials of glass, 7 cm long and 16 mm in inside diameter, which were closed at the top by gauze attached by a rubber band. These shell vials were arranged on a wooden rack in a semicircle around the radium needle at a distance of 27.5 cm. According to calculation the dose received in this way would be 2.5 r/day. This value was checked by William F. Bale with a Victoreen dosimeter, and a value of 3.3 r/day was found. A later check performed by the authors with a different Victoreen dosimeter gave 2.3 r/day. Within the error of the measurements it seems, therefore, that the theoretical value of 2.5 r/day is correct.

During the experiment the flies, Drosophila melanogaster, were kept in an incubator of Celotex which was cooled by a compressor unit driven by a ⅓-hp motor and heated by two 60-watt bulbs controlled by a thermostat. In this manner the temperature was kept constant at 18 ± 1°C.

The controls were kept in an incubator of the same size and type at 18 ± 1°C. The control incubator was cooled by the same compressor as the experimental incubator so that any disturbance in the compressor unit would affect both experimentals and controls in a similar way. The only possibility of a difference between experimentals and controls was a failure of the bulbs used as heating units. For this reason, both incubators were checked twice each day to determine whether the bulbs were still working.

Since the experimental and control incubators were in the same room, the control incubator was protected from the gamma rays of the experimental incubator by means of a lead plate 5 cm thick which was attached at a distance of 4 cm in the back of the radium needle. The distance between the two incubators was 1.4 meters. Radiation in the control incubator was checked by Bale with a Victoreen dosimeter and found to be 0.03 r/24 hr, i.e., 1 per cent of the radiation that the experimentals received. The control flies were kept in glass shell vials, identical with those for the experimentals, and placed in the incubator on a semicircular rack, identical with that used for the experimentals except that no radium needle was present.

The breeding procedure was similar to that used by Spencer and Stern, and the effect studied was identical with theirs, i.e., lethal mutations in the X chromosome of the Canton-Special strain. For purposes of testing, this strain was bred to the Muller-5 strain, which also was used by Spencer and Stern. The Muller 5 has an X chromosome marked by the dominant gene Bar (B) and the recessive gene apricot (w^a). Crossing over with the homologous Canton-Special chromosome is inhibited by two inversions in the Muller-5 X chromosome, one of which includes the other.

Virgin Muller-5 females were isolated every day. Fifty females were mated to one hundred Canton-Special males in ½-pint bottles. During most of the course of the experiment, males had been mated before for at least 5 days to their Canton-Special sisters. This was done because H. J. Muller had advised that his results indicated that the spontaneous mutation rate of the first sperm discharged by a young male is higher than in the sperm formed later in life. Although the first experiments were done on males which had not been aged, there was no error introduced by this fact into the experiment since it was identical for both experimentals and controls. Actually no significant difference was found between the control mutation rates in the first experiments, in which the age of the males was not controlled, and in the later experiments, in which aged males were used.

The Muller-5 females were allowed to remain with Canton-Special males for 4 days. After they had been inseminated, carrying Canton-Special sperm in their spermathecae, they were etherized and prepared for irradiation. The preparation for irradiation consisted in placing the females into the above-mentioned shell vials, which contained food in the form of a specially prepared honey-yeast agar slant and a piece of tissue paper to absorb excess moisture. The vials, each containing 50 female Muller-5 flies with Canton-Special sperm in their spermathecae, were then transferred to the incubators. One-half the vials were placed into the control incubator and one-half into the experimental incubator. In placing the vials into the experimental incubator, care was taken that the agar slant was directed toward the outside, away from the radium, so that the gamma rays did not have to penetrate the agar before reaching the flies.

The flies were exposed to the radium in the incubator for 21 days; thus the total dose of radiation received was 52.5 r. The dose received by the controls in the same interval was about 0.6 r. During these 21 days the food was changed once, both in the experimentals and the controls, by shaking the flies without etherization into new shell vials containing newly made up medium, on the tenth or eleventh day.

The choice of honey-yeast agar was made after preliminary tests with several media. The medium required was one that would inhibit the flies from laying eggs but still allow them to survive. The survival was increased by keeping them at the low temperature of 18°C; the egg-laying was inhibited to a certain degree by relatively dry conditions induced by the addition of tissue paper and by the use of gauze in closing the shell vials. This precaution was necessary in order to inhibit the flies from expending the sperm stored in their spermathecae.

Table 15.1 — Sterility and Fertility of Irradiated Females Aged for 21 Days at 18°C on Different Media

	Honey agar	Molasses agar	Honey-yeast agar	Cornmeal-molasses agar
Sterile females, %	37.7 ± 1.9	59.0 ± 4.9	31.0 ± 1.7	42.2 ± 2.7
Average number of F_1 females per culture	8.97 ± 0.71	12.12 ± 1.44	12.10 ± 0.42	19.37 ± 1.27
Average number of F_1 males per culture	4.57 ± 0.45	5.08 ± 0.69	6.06 ± 0.24	7.07 ± 0.92

Four different media were tried: molasses agar, honey agar, yeasted honey agar, and yeasted cornmeal-molasses agar. The results obtained with these four media are given in Table 15.1. Survival of the flies was not included, but it should be stated that with every medium 50 to 80 per cent of the flies were lost, partly by death and partly by escape while the medium was being changed.

After having been aged on one of the four media for 21 days at 18°C without irradiation, the surviving females were etherized and put singly into 1-oz creamers containing yeasted cornmeal-molasses agar. These creamers were kept at 25°C for 2 weeks, and the failures, as well as the flies hatching in the single fertile creamers, were counted.

As far as the ability of females to produce any offspring is concerned, honey-yeast agar was superior to the three other media used. The highest fecundity, as judged by the number of offspring, was found after aging on cornmeal-molasses agar. Since cornmeal-molasses agar offered better conditions for the development of molds during the aging processes and since only a limited number of flies from each culture was desired for every breeding experiment, yeasted honey agar, containing 10 g of carrageen, ½ pint of honey, dry yeast, and Moldex in 1 qt of water, was used as the aging medium.

A few remarks are pertinent concerning the causes of the high sterility on all media and the reduced fecundity as compared with nonaged flies. The question of fertility of Drosophila females at different ages has been investigated by Hadorn and Zeller.[8] They found a notable decrease in fecundity in older females; this decrease was proved to be due to the physiological state of the female and not to exhaustion of sperm. It is in agreement with their findings that, in the experiments in this laboratory, the laying of eggs in the aging vials was never high; therefore the stored sperm was certainly not exhausted. Females of 21 days of age, according to Hadorn and Zeller, are far advanced toward infertility. This is expressed by a decrease of the number of eggs laid by about 40 per cent at 20 days and by a rate of hatching of the eggs of less than 20 per cent, as compared to 60 to 80 per cent in the first 2 weeks.

After having been aged and irradiated for 21 days, the surviving females were tested for sex-linked recessive lethals, according to the breeding scheme indicated in Fig. 15.1.

The aged and irradiated females were put separately into 1- or ¾-oz creamers containing the usual Drosophila food medium, yeasted cornmeal-molasses agar with Moldex added. They were kept for 2 weeks at a temperature of 24°C. At the end of this period, their progeny had developed. The males had the Muller-5 X chromosome from their mother and showed the Muller-5 phenotype, apricot and Bar. The females had X chromosomes from both their Canton-Special fathers and their Muller-5 mothers. Phenotypically they had wild-type eye color and the kidney-shaped eyes characteristic of heterozygous Bar.

As indicated in Table 15.1, a large percentage of the cultures was sterile. The number of offspring obtained in different cultures varied. The breeding of isolated aged and irradiated females permitted the study of the influence of radiation on sterility and fecundity. It also had some advantages in diminishing the error introduced by the phenomenon of lethal clusters. This question is discussed in Sec. 4.

When the F_1 animals had hatched, pair matings were made between F_1 females and their brothers or, since there were always fewer males than females, with Muller-5 males from stock cultures. Not more than 14 females from any F_1 culture were used for mating, another precaution that reduced the error introduced by lethal clusters, as is discussed in Sec. 4.

The pair matings were made up in ¾- or 1-oz creamers with yeasted cornmeal medium just as with the F_1 culture. The creamers were kept on trays. Since the size of both the trays and the creamers varied, the number of cultures per tray varied from 35 to 165. The cultures were kept in a constant-temperature cabinet at 25 ± 1°C. The cabinet, as suggested by Warren P. Spencer, consisted of five sections of shelves, 212 cm high, 93 cm wide, and 30 cm deep. One of these sections was placed against a wall, and the other four formed the sidewalls of the cabinet. They were roofed over with Celotex and were insulated toward the outside by a layer of Celotex. The front wall was also made of Celotex and contained a wooden door. The space inside the cabinet was about 5½ by 6 ft; the shelves opened into this space. The cabinet was kept at 25°C by means of a heating element inserted into a motor-driven blower which was regulated by a thermostat.

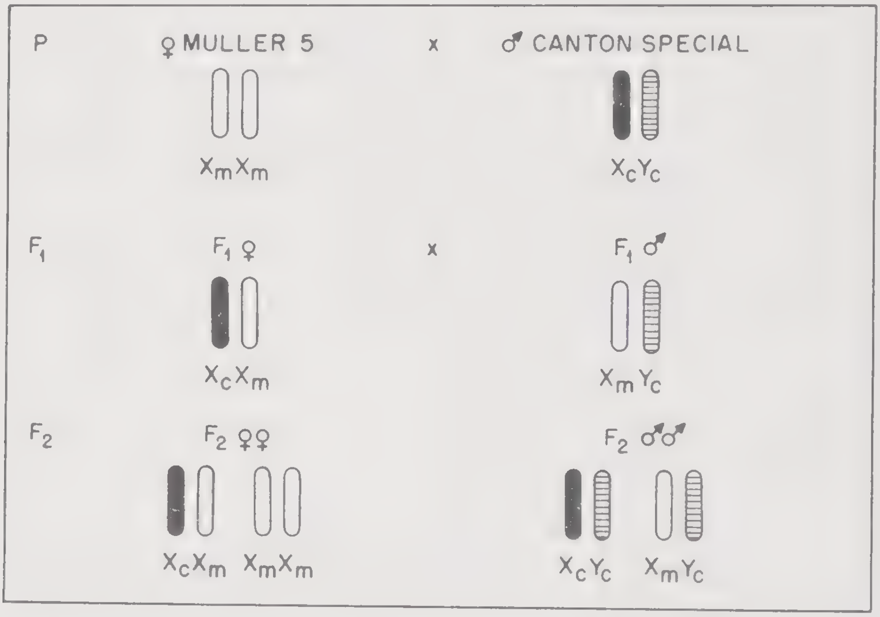

Fig. 15.1—Breeding scheme for Drosophila melanogaster.

Keeping the cultures at a constant temperature had two advantages. It kept the cultures at a steady speed of development so that they were ready to be examined 14 days after they had been made up. Furthermore, the expression of lethals is dependent on external conditions; the same gene may act as a complete lethal under one set of conditions, whereas, under another set of conditions, some animals carrying the lethal will survive. Control of temperature will therefore tend to standardize environmental conditions and make the results more comparable.

After 14 days the hatching cultures were examined. A number of F_2 cultures (varying from 0 to over 60 per cent, but usually around 10 per cent) were sterile. In the viable cultures, four types of progeny are expected, as indicated in Fig. 15.1. Of these, the phenotypically wild-type males carry the original Canton-Special chromosome in hemizygous condition. If a recessive lethal mutation arises in this chromosome, these males should die at some stage of development and therefore should be missing in the F_2 cultures.

In order to find the lethals the cultures were therefore examined for the presence of wild-type males. If, by simple inspection with a binocular microscope through the walls of the culture jar, wild-type males were seen, the culture was counted as not lethal. If no wild-type males were found, the flies in the culture were etherized, and the flies belonging to the different classes were counted. This count was repeated twice at 2- to 3-day intervals. If no wild-type males appeared during this time, two females heterozygous for the lethal-carrying wild-type chromosome were mated in pair matings to their Muller-5 brothers. These pair-mating cultures were kept for 2 weeks in the constant-temperature cabinet; then the different classes of flies hatching in the progeny during the third week were counted. The X chromosome was considered as carrying a lethal if no wild-type males appeared in the F_2 culture, even though in some cases a small number of wild-type males appeared in the following generation. If, on the other hand, a single wild-type male appeared in the F_2 culture, the culture was not counted as a lethal but as a semilethal, regardless of whether or not both cultures in the next generation contained any wild-type males. The only exception to this rule was made when the F_2 culture contained less than 50 flies. In this case it is possible that no wild-type males are found, purely by chance; therefore the decision as to whether a culture should be considered as lethal or not lethal was made on the basis of the appearance of wild-type males in the next generation.

These criteria are admittedly arbitrary. But since there exist all intergrades between full viability, lowered viability, semilethality, and complete lethality and since the expression of these characters is also dependent on environmental conditions, distinct criteria had to be set up which were applied to controls and experimentals alike. In the actual experiments, however, most lethals proved to be clear-cut, and only rarely was it necessary to make a decision according to the above-mentioned criteria.

Once a lethal was established, it was kept by mating several females, that carried the lethal, to their Muller-5 brothers. The lethals were kept in ½-pint milk bottles with yeasted cornmeal-molasses agar and were later used for linkage tests.

3. RESULTS*

The results obtained are indicated in Tables 15.2 to 15.5. Table 15.2 contains the sterility in percentages and the average number of males and females produced by females aged for 21 days and by females irradiated during this period with gamma rays amounting to 52.5 r. The data do not indicate any significant difference between the experimentals and the controls. There is therefore no evidence that the fertility of the females has been affected by the irradiation.

Table 15.2 — Sterility and Fertility of Females*

	Controls	Number of cultures	Experimentals	Number of cultures
Sterile females, %	41.1 ± 0.25	3988	40.7 ± 0.24	4002
Mean number of females per culture	19.7 ± 0.9	187	19.3 ± 0.8	261
Mean number of males per culture	10.7 ± 0.5	187	11.6 ± 0.5	261

*Controls were aged for 21 days at 18°C, and the experimentals were irradiated for 21 days at 18°C with gamma rays amounting to 52.5 r.

The lethal mutation rates in the experimentals and in the controls are given in Table 15.3. The results of Spencer and Stern, obtained from nonaged sperm and from sperm irradiated with X rays of 50 r, are included in the table. The last column gives the percentage values of which the observed mutation rates would be the upper and lower

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* These results and the discussion following should be considered in the light of subsequent experiments of Uphoff and Stern which are summarized in the Appendix to this chapter.

5 per cent fiducial limits (5 per cent in both tails jointly). These were calculated according to a formula proposed by D. R. Charles, as reported by Spencer and Stern, and according to the procedure of Stevens.[9] It is seen, by comparing lines 1 and 3, that the control mutation rate in Spencer and Stern's material was lower than in the experiments reported here. This is due to the aging effect. This phenomenon had been described previously by Rajewski and Timoféeff-Ressovsky.[10] By inhibiting males from mating for 20 days, they were able to increase the mutation rate from 0.104 per cent in sperm used for fertilization shortly after hatching to 0.263 per cent in sperm aged 20 days. These values are in good agreement with those found in this experiment. Since, in this experiment, the sperm were kept in the female spermatheca while aging, the increase of mutation rate seems to be due to aging rather than to place of storage.

Table 15.3 — Lethal Mutation Rate in the X Chromosome of Canton-Special Sperm After Different Types of Treatment

Treatment	Number of cultures tested	Number of lethals found	Lethals, %	Calculated limits of lethals, % (see text)
Controls aged	56,252	154	0.2738	0.2356 – 0.3198
52.5-r chronic exposure	51,963	162	0.3118	0.2692 – 0.3628
Controls not aged*	73,901	72	0.0974	0.0788 – 0.1220
50-r acute exposure*	31,560	77	0.2440	0.1985 – 0.3034

*These values are taken from Spencer and Stern.

The comparison of the mutation rate of sperm irradiated with 52.5 r chronically and of sperm of the aged controls shows that it is somewhat increased in the experimentals. This increase is, however, slight and far inside the 5 per cent level of significance. Therefore in these data there is no indication that the difference observed between experimentals and controls is not due to chance alone.

This result seems to be in direct contradiction to Spencer and Stern's finding that acute irradiation with 50- and even 25-r X rays causes a significant increase in mutation rate. Since the controls of the two experiments have very different mutation rates owing to the aging effect, the experimental mutation rates cannot be directly compared. Only the difference between the mutation rates in experimentals and controls forms a legitimate basis for the comparison of Spencer and Stern's experiments with those reported here.

The difference observed by Spencer and Stern between the mutation rates in sperm receiving an acute dose of X rays of 50 r and their controls is 0.1466 per cent. The corresponding difference in the experiments described in this chapter is 0.0380 per cent. It is necessary to calculate what the probability is that such a difference might occur by chance.

If it is assumed that the two differences are not significantly different from each other, they must be considered as chance deviations from a universe of differences. As a best estimate for this universe, from which the two values are chance deviations, D. R. Charles has suggested the expression

Table 15.4 — Test for Homogeneity of Differences Between Experimentals and Controls

	Experimentals		Controls	
	Lethals	Nonlethals	Lethals	Nonlethals
Spencer and Stern				
Expected	66.15	31,493.85	82.85	73.818.15
Observed	77	31,483	72	73,829
Caspari and Stern				
Expected	178.1	51,784.9	137.9	56,014.1
Observed	162	51,801	154	55,998

$$\frac{\dfrac{D_1}{(SED_1)^2} + \dfrac{D_2}{(SED_2)^2}}{\dfrac{1}{(SED_1)^2} + \dfrac{1}{(SED_2)^2}}$$

in which D_1 and D_2 represent the differences between experimentals and controls found in this work and in Spencer and Stern's experiments, respectively, and SED_1 and SED_2 represent their respective standard errors. By introducing the actual values observed, a value for the best estimate of the difference of 0.0975 per cent is obtained.

Taking this value as the most probable difference, expectations can be calculated for the number of lethals and nonlethals to be found in the experimentals and controls in the two experiments. In this way eight values for expectations are obtained for the lethals and nonlethals in experimentals and controls in both experiments. These are compared with values actually obtained by a chi-square test (Table 15.4).

In this way a chi square of 6.1523 for 1 deg of freedom is obtained. This corresponds to a probability of about 0.012. In other words, there is about 1 chance in 83 that the two differences are chance deviations from the same sample. This probability is at a level which is usually regarded as significant.

In Table 15.5 the mutation rates obtained in experimentals and controls during the different months of the experiment are given. Since experimentals and controls were reared and treated side by side, the results obtained in each month can be compared. The monthly mutation rates appear highly variable both in the experimentals and in the controls. In five out of eight months the experimentals showed a higher mutation rate than the controls, and in the remaining three months the controls had the higher rate. In three of the months the difference between the mutation rates was of the order expected from Spencer and Stern's results, but in one of these months the estimate for the experimentals is certainly too high because of the occurrence of a large lethal cluster (see Sec. 4). In three months the difference between experimentals and controls was very slight, whereas in the remaining two (April and May) the mutation rate was somewhat higher in the controls. From the comparison of mutation rates in the different months, no conclusion can be drawn concerning the effect of the radiation. An effect of radiation on mutation rate does not seem to be excluded, but it is certainly overshadowed by the chance fluctuations from month to month.

Table 15.5 — Lethal Mutation Rate in the X Chromosome of Canton-Special Sperm

	Experimentals			Controls			
Month	No. of tests	No. of lethals	Lethals, %	No. of tests	No. of lethals	Lethals, %	Difference*
October				7022	16	0.2279	
November	6715	22	0.3276	5320	11	0.2068	+0.1208
December	6441	14	0.2174	6008	14	0.2330	−0.0156
January	5223	10	0.1915	5466	9	0.1647	+0.0268
February	7694	31†	0.4029	6626	17	0.2566	+0.1463
March	6540	26	0.3976	5974	15	0.2511	+0.1465
April	7165	15	0.2094	6414	18	0.2806	−0.0712
May	6770	23	0.3397	6772	29	0.4282	−0.0885
June	5415	21	0.3878	6650	25	0.3759	+0.0119

* Difference between experimentals and controls.

† Lethal cluster of nine lethals included.

4. IMPORTANCE OF LETHAL CLUSTERS

The phrase "lethal clusters" is used to designate the occurrence of a number of lethals in the progeny from the same mating. It is interpreted as indicating the occurrence of a lethal mutation early in the course of spermatogenesis. If a lethal mutation occurs in an early spermatogonium, all X-bearing sperms derived from this spermatogonium will contain the lethal and transmit it to the next generation. The earlier in spermatogenesis the mutation occurs, the larger will be the number of cultures in the F_2 generation which will form a lethal cluster.

Lethal clusters have been disregarded in earlier work on mutations since they are negligible compared to the high mutation rate induced by radiation at high dosages, but in working with low mutation rates this effect cannot be disregarded since, if an absolute number of mutations of about 150 is compared, two or three lethal clusters of three or four lethals or one larger lethal cluster may disturb the results considerably. For this reason Spencer and Stern have considered this phenomenon in their experiments at low dosages and discussed its occurrence.

In the investigation reported in this chapter, precautions have been taken to avoid the danger of lethal clusters. The irradiated P females were isolated, and not more than 14 of the F_1 daughters of each female were tested for lethals. Actually, the number was frequently even lower, since in many F_1 cultures less than 10 females were produced. This procedure limits the size of any lethal cluster to 14 at the most.

Furthermore, the individual F_1 daughters of the same irradiated female were always placed in neighboring creamers on the same tray. This arrangement would immediately call attention to the occurrence of a lethal cluster. Since, in the experiments at a mutation rate of 0.25 to 0.3, on the average only one lethal was found on three to four trays examined, a large number of lethals on the same tray and in the same neighborhood would always arouse suspicion.

Finally, unless some extremely large lethal clusters occurred, the rate of lethal clusters in experimentals and controls should be similar since mature sperm were irradiated in the spermatheca of the female. This is a difference between the experiments described in this chapter and those of Spencer and Stern. Spencer and Stern irradiated males which carried both sperm and spermatogonia. A lethal mutation induced by radiation in a spermatogonium would give rise to a cluster of lethals. Since, in the experiments being reported, no spermatogonia were irradiated, no lethal clusters could have been produced by radiation. All lethal clusters, both in controls and in experimentals, must have been produced before the aging and irradiation of the sperm.

The actual frequency of occurrence of lethal clusters can be estimated. Designating as a "lethal association" all cases in which two or more lethals were found on the same tray, the question arises as to how many associations of two, three, four, etc., lethals will be expected to occur by chance, taking the lethal mutation rates from Table 15.3 and the averages of 84.5 fertile cultures per tray for the experimentals and 85.2 cultures per tray for the controls. In this way expectations for the occurrence of two, three, etc., lethals on the same tray can be derived from the formula for the Poisson series.

Table 15.6 — Comparison of Expectations for Chance Occurrence of Different Numbers of Lethals to the Actual Numbers Observed

Number of lethals	Frequency of occurrence: Controls, Expected	Controls, Observed	Experimentals, Expected	Experimentals, Observed
1	107.68	87	124.48	101
2	12.56	18	16.40	17
3	0.978	3	1.44	3
4	0.057	1	0.095	1
5		0	0.005	1
6		0		0
7		0		0
8		0		0
9		0	7.95×10^{-9}	1

In Table 15.6 the expectations calculated in this way are compared with the distribution of lethals on the trays actually observed. Although in the experimentals the whole material has been used, the control data are based on 49,700 observations (i.e., 88.35 per cent of the total), among which there were 136 lethals (mutation rate 0.2738 per cent). This is due to the fact that, in the beginning of the experiments in which only controls were tested, the distribution of lethals on the trays was not recorded.

From Table 15.6 it appears that in the experimentals there occurred one lethal cluster of nine. Its occurrence by chance is highly improbable. Actually, these lethals occurred in two neighboring rows on the same tray, i.e., the nine lethals were found in one sample of

27 cultures. It seems very likely that they constitute one mutational step which occurred early in spermatogonial development. The two lethal associations of five and four lethals each may also be lethal clusters. There is no reason to assume anything other than chance association for the occurrence of two- and three-lethal clusters on one tray, although one or two of these groups may also constitute lethal clusters. Linkage tests, described below, suggest that some of the associations of two and three lethals represented clusters.

In the controls there are no unexpectedly high associations on the trays, except for one tray with four lethals which may possibly constitute a lethal cluster. Otherwise, there may be some lethal clusters of three or two lethals involved. This is suggested by the results of the linkage tests. This difference between experimentals and controls must be due entirely to chance since the mutational steps in question must have occurred before irradiation.

It is not legitimate to correct for the lethal clusters derived in this way. But it may be suggested that, if the groups of four, five, and nine lethals are regarded as one mutational step each, a decrease in experimental mutation rate from 162 in 51,963 to 147 in 51,963 (0.2829 per cent) would be obtained. If the controls were corrected in a similar manner, assuming as a safety factor that the association of four lethals, two of the associations of three lethals, and six of the associations of two lethals are lethal clusters, a corrected lethal mutation rate of 123 in 49,700 tests (0.2475 per cent) would be obtained. The difference, 0.0354 per cent, would be of the same order of magnitude as in the uncorrected data.

Although this attempt at correcting for lethal clusters may appear to be illegitimate, it serves well the purpose of demonstrating that these corrections will not increase the difference between experimentals and controls since the larger lethal clusters were found in the experimentals.

Another method by which the clustering of lethals may be estimated is the determination of their position on the chromosome. If two lethals are differently situated on the chromosome, they must be due to independent mutation events. If they are at the same place, they may or may not be identical mutations. For this reason a number of mutations which had arisen on the same tray were tested by linkage experiments. Females heterozygous for the lethal were crossed out to males from a stock whose X chromosome was marked by the genes sc, cv, v, f, and car. F_1 females were mated singly in creamers to their brothers. In F_2 cultures the different recombinations appearing among the males were counted. On the whole, 35 control lethals and 36 experimentals have been examined by linkage tests.

The linkage experiments did not yield any unexpected results. As in the linkage experiments of Spencer and Stern, the average map length in these experiments was somewhat larger than in the Bridges map, mainly because the cv-v segment was increased. An unexpected increase of certain map regions was observed in some of the lethals in both experimentals and controls. This same phenomenon is described by Spencer and Stern. No interpretation can be given. In any case, it cannot be a result of the irradiation since it also occurred in the controls.

One control lethal, l_c94, showed a considerable shortening of the map. It measured 40.2 units, as compared with 67.5 units in Spencer and Stern's standard map. This would seem to suggest the possible occurrence of an inversion. Since it occurred in a control, it would constitute a spontaneous inversion. Unfortunately, the stock was lost; therefore no cytological tests could be carried out.

In the experimentals the large lethal cluster of nine was investigated by linkage tests. Tests on eight of the nine lethals showed that the lethal was situated between cv and v, 11.4 to 15.7 units to the right of crossveinless. One of the lethals of this cluster was lost.

In the experimental lethal association of five lethals, all were situated between v and f. Four of them were only slightly to the left of f, between 1.9 and 6.5 units, and the fifth was situated much farther to the left from f, 17.0 units. The four lethals near f may well constitute a lethal cluster, but it seems doubtful that the fifth one belongs to it. Of the lethal association of four lethals, three were all situated in the short region between f and cv, and the fourth was lost. In one of the three associations of three lethals each, all three lethals were situated in the same region of the chromosome, whereas, in the other two associations of three lethals, only two lethals were investigated; the third was lost. In both cases the two lethals were in the same region of the chromosome. Of the 17 associations of two lethals, 12 were investigated. Seven of these were far removed from each other; thus they could not possibly be derived from the same mutated cell. The remaining five associations contained two lethals each in the same region of the chromosome.

Of the control lethals, the association of four lethals was tested. One of the four was lost; the remaining three were in the same region of the chromosome, 9.7, 9.0, and 5.4 units to the right of v, respectively. The control lethal associations of three lethals each had two of the lethals in the same region of the chromosome, and the third was in all cases definitely removed from it. Finally, 12 of the 18 lethal associations of two lethals each were investigated. Six pairs of them were certainly not identical mutations, and the other six pairs were near enough to allow the possibility that they may have been a lethal cluster.

These observations bear out the conclusion, drawn from statistical considerations, that the experimental association of nine and four lethals constitute a lethal cluster, i.e., that each one constitutes only one mutational step. The same is true for at least four lethals from the association of five, and the fifth remains doubtful. With the associations of two lethal-carrying creamers on the same tray, the same conclusion is not justified. Although occurrence in different regions of the chromosome excludes the possibility that the two lethals may be due to the same mutational step, occurrence in the same region does not prove it. This is well indicated by the two control lethals l_c29 and l_c30. Although they are both situated slightly to the right of v, the death in l_c30 occurs in the pupal period, whereas in l_c29 it occurs earlier. This difference in developmental effect excludes the possibility that both lethals may be due to the same mutation.

A reliable estimate of the actual influence of lethal clustering is not possible with either of the methods used, but it must be concluded that correction for lethal clustering will not tend to increase the difference between experimentals and controls. Suppose that in the controls (Table 15.6) all associations of lethals are counted as clusters but that in the experimentals only the clusters of nine and four (confirmed by linkage tests) are corrected for. The difference between experimentals and controls still would be only 0.0443 per cent, i.e., much lower than the difference found by Spencer and Stern at 50 r. Since the assumption that all lethal associations in the controls were due to clusters is highly unlikely, it must be concluded that the difference found in the original experiments probably approximates the real difference.

5. DISCUSSION

The results show with a high degree of probability that the mutation rate obtained in the investigation reported in this chapter is lower than that obtained by Spencer and Stern on the same strain with the same dose of radiation. In order to explain this difference, all differences in experimental technique between Spencer and Stern's and these investigations will be discussed separately. These differences are (a) temperature, (b) aging, (c) wavelength, (d) intensity of radiation, and (e) plastic vs. glass containers.

5.1 Temperature. In the experiments reported in this chapter the flies were kept at 18°C during the irradiation, whereas Spencer and

Stern's irradiations were done at room temperature, i.e., probably at around 24°C. Since temperature is known to affect many biological processes, this factor cannot be neglected. The influence of temperature on radiation-induced mutations has been studied repeatedly. Timoféeff-Ressovsky[11] and Muller,[12] working with temperatures of 10 against 35°C and 5 against 37°C, respectively, did not find any difference in mutation rate induced by X rays. King[13] obtained an increase in mutation rate at 0°C as compared to room temperature.

It therefore seems unlikely that the lower mutation rate obtained in this experiment is due to the lowering of the temperature to 18°C. Besides, in order to explain the difference obtained in the two mutation rates that were found, a Q_{10} of about 4 must be assumed. In photochemical processes the Q_{10} usually found lies between 1.01 and 1.07. If, therefore, the temperature difference should be responsible for the effect observed, secondary processes with a high Q_{10} would have to be postulated. It should be stated that Timoféeff-Ressovsky[14] has found a similarly high Q_{10} (5.7 for D. melanogaster, 3.00 for D. funebris) for "spontaneous" mutations.

5.2 Aging. In the Spencer and Stern experiments on irradiated males, the sperm were used for fertilization immediately after irradiation. In the experiments reported here, the sperm were stored in the spermathecae of the females and therefore were subject during irradiation to an aging process. That this aging process in itself may actually influence mutation rate is indicated by the excess of control mutation in our experiments as compared to those of Spencer and Stern.

To explain the difference in frequency of induced mutations between Spencer and Stern's results and our results by the difference in the age of sperm would imply that the physiological state of the sperm cell influences the mutation rate under the influence of radiation. There are actually data in the literature which seem to prove this assumption.

Knapp and Kaplan[15,16] increased the mutation rate induced by X rays in eggs and pollen of Antirrhinum majus by immersion in water. They explain this by the assumption that the amount of hydration of the cell colloids influenced the mutation rate induced by X rays. This is borne out by the fact that the effect can be reversed by drying the seeds after hydration. Hollaender and Swanson[17] found an increase in the X-ray-induced mutation rate of Aspergillus by pretreatment with infrared rays. Kaufmann,[18] on the other hand, did not obtain an increase of X-ray-induced sex-linked mutations in Drosophila after pretreatment with infrared rays. These investigations leave no doubt that the physiological state of the irradiated cell may influence the rate of transmissible changes produced by radiation. There is therefore no reason to exclude a priori the possibility that the aging of sperm cells in the spermatheca reduces the mutation rate produced by irradiation.

Offermann[19] has claimed to have found this effect in Drosophila, i.e., an increase in X-ray-induced mutation rate in aged as compared to nonaged Drosophila sperm, whereas Timoféeff-Ressovsky[20] did not find any influence of aging on X-ray-induced mutation rate in Drosophila sperm. These experiments seem to indicate that the effect of aging on induced mutation rate would result in an increase of mutation rate rather than a decrease. It seems doubtful, however, that the aging process in seeds and pollen in air and of sperm in the male is physiologically equivalent to aging in the spermatheca of the female.

5.3 Wavelength. The experiments reported in this chapter were carried out with gamma rays, whereas Spencer and Stern worked with X rays. The number of ionizations produced in this way was equal as expressed by the number of roentgens. A difference between these two types of radiation could possibly arise in two different ways. Gamma-ray quanta have a higher energy than X-ray quanta, and therefore a lower number of quanta is absorbed to produce the same effect as expressed in number of ionizations. Furthermore, the pattern of ionizations will be different; gamma rays produce a more scattered track of ionizations than X rays.

A consequence of the different spacing of ionizations due to X rays and gamma rays is that the number of cells subject to ionizations will be smaller, owing to the fact that with the use of X rays the ionizations tend to occur close to each other. Assuming that one ionization in a sensitive volume is sufficient to cause a mutation, the mutation rate produced will be dependent on the number of ionizations per cell. The number of ionization events per cell will therefore be compared for Spencer and Stern's experiment and for this experiment.

In both cases about 50 r was given. Since 1 r corresponds to the production of 1.62×10^{12} ion pairs per gram of tissue, 50 r would produce 81×10^{12} ion pairs per gram of tissue. According to Gowen and Gay[21] the sperm head of Drosophila is 7.36×10^{-4} cm long and 0.368×10^{-4} cm wide. Its weight would therefore be in the neighborhood of 1×10^{-12} g. This would lead to the conclusion that, both in Spencer and Stern's experiment and in these experiments, on the average 81 ion pairs were produced in each sperm head.

The specific ionization of gamma rays and medium-soft X rays is different. X rays of 4.1 A form 2.7×10^{6} ion pairs per centimeter of track, whereas gamma rays form 1.5×10^{5} ion pairs per centimeter of track.[22] This would mean 270 and 15 ion pairs per micron, respectively. The consequence would be that, if the mutation rate induced is to be dependent on hitting a certain sensitive volume, the total number of ionizations being the same, the chances are better with the use of gamma rays than with the use of X rays. Owing to the closer spacing of the ion pairs in X rays, a number of X rays would be wasted by falling into an identical sensitive volume. This consideration would lead to the consequence that gamma rays should be more efficient rather than less efficient than X rays, which is contrary to the results.

The possibility remains that the difference between the results obtained by Spencer and Stern and those in this investigation is due to differential absorptions of quanta. Since one quantum of gamma rays contains more energy than one quantum of X rays, a smaller number is absorbed to cause the same amount of ionization, expressed in roentgens. If a mutation was dependent on the absorption of one quantum, X rays should therefore be more effective than gamma rays, but this conclusion is contradicted by all experiments performed at higher dosages, in which no differences in mutation rates, produced by the same dosage of X rays of different wavelength and of gamma rays, were found.[12,23]

A third possibility must be taken into consideration: the higher absorption of X rays as compared with gamma rays by elements of high atomic weight. Gray[24] has studied this effect for sulfur and has explained the greater effect of X rays over gamma rays of the same dose in roentgens, which was found by Mottram and Gray[25] for lesions of the skin in the mouse induced by radiation, by the higher absorption of X rays in the keratin, which is rich in sulfur. In radiation effects on genes a similar effect must be seriously considered since chromosomes are rich in phosphorus. According to Mirsky and Pollister,[26] sperm of different species contains 3 to 6 per cent phosphorus. Because of the high absorption of radiation by the heavy phosphorus atoms, the actual radiation received by irradiated sperm heads should be considerably higher than that usually estimated for soft tissue. However, no experimental evidence for the higher absorption effect of phosphorus atoms on X rays has been found in experiments with high dosages.

5.4 Intensity. In Spencer and Stern's experiment the flies were exposed to the entire dose in a short time; in the experiments described here the same dose was spread over 21 days. The intensity of radiation per minute was about $\frac{1}{6000}$ to $\frac{1}{13{,}200}$ of that used by Spencer and Stern, dependent on whether a dose of 10 or 22 r/min was given by Spencer and Stern.

Influence of intensity on the effect of a radiation has been reported for numerous effects such as the killing of bacteria and animal eggs. In all these cases, sigmoid killing curves have been obtained. This is in contrast to the effects previously obtained in experiments on mutations, in which a direct proportionality between dose and mutation rate was always found, independently of the time factor.

This difference between "physiological" radiation effects and mutation effect has been explained by the assumption of the presence or the absence of recovery.

It is assumed that in physiological effects the injury caused by the primary ionizations can be compensated for if the original injury is sufficiently small and if sufficient time is at the disposal of the cell. It is conceivable that the same is true for radiation injury, and the dosage which makes recovery possible has to be as low as $\frac{1}{600}$ r/min. This possibility and its implications are discussed in Sec. 5.5.

5.5 Plastic vs. Glass Containers. It must be considered that Spencer and Stern kept their flies in plastic containers during the irradiation, whereas, in the experiments reported in this chapter, glass shell vials were used. It is probable that, because of their silica content, the glass vials would absorb more radiation than the plastic vials.

In order to obtain an estimate of the amount of the radiation that was being absorbed by the glass walls, ionization chambers of the shape and size of a fountain pen were exposed to the gamma rays used in the experiments inside and outside a test tube having a wall thickness similar to that of the shell vials. Five different ionization chambers were used, and they were exposed for either 30 or 60 min. Fifteen measurements were made outside the glass test tube, and seven measurements were made inside the tube. The results obtained with the different dosimeters were in close agreement with each other.

With the ionization chambers exposed directly to the gamma rays, a mean value of 0.097 ± 0.002 r/hr was found, although, from the weight of the radium and the distance, a dose of 0.104 r/hr would be expected. The ionization chambers contained in glass tubes showed a mean value of 0.084 ± 0.002 r/hr, 0.013 r/hr less than the unprotected ionization chambers. This would point to an absorption of 13.4 per cent of the radiation by the glass wall. The actual dosage received by the flies was therefore about 45 rather than 50 r. This correction is not being used in this chapter since similar corrections have not been obtained for Spencer and Stern's data or for the other data described in the literature. Spencer and Stern's plastic capsules were probably less absorbing, but their soft X rays may have been more readily absorbed than the highly penetrating gamma rays. At all events the absorption by the glass is certainly not sufficient to account for the difference between Spencer and Stern's experiment and the one reported here.

Although the analysis up to this point excludes the possibility that differential absorption by the container may have been responsible for the different results obtained by Spencer and Stern and by us, the other possibilities suggested are still more or less likely.

A further analysis is possible by comparing the data reported here to those of Raychaudhuri.[6,27] He irradiated inseminated Drosophila females with low doses of gamma rays for 30 days at 8°C and studied the production of sex-linked lethal mutations by a breeding procedure similar to that used in this experiment. The results obtained by him and by Muller[12] are given in Table 15.7.

Table 15.7 — Lethal Mutations Induced in the X Chromosome of Drosophila by X Rays and Gamma Rays at Different Intensities*

Type of radiation	Dosage, r/min	Time of exposure	Total dosage, r	Number of cultures	Number of lethals	% lethals ±SE
X rays	250.00	8 min	~2000	6224	373	5.99 ± 0.27
γ rays	0.01	720 hr	400	3855	59	1.53 ± 0.20
γ rays	0.05	720 hr	2000	868	58	6.675 ± 0.85
γ rays	0.85	45 hr	1300	762	40	5.25 ± 0.82
None				3471	11	0.317 ± 0.095

*Taken from references 6, 12, and 27.

The data given in Table 15.7 present a picture quite different from that obtained in this study. In both cases 2000 r produced mutation rates around 6 per cent, regardless of whether the radiation was given as X rays or gamma rays and in 30 days or in 8 min. Furthermore, 400 r produced roughly one-fifth the mutation rate at 2000 r. The results of Muller and of Raychaudhuri are therefore in complete agreement with the rule that mutation rate is directly proportional to the dosage as measured in roentgens and is independent of the wavelength employed and of the intensity.

Since Raychaudhuri's experiments were executed with a technique very similar to that used in the studies described here, certain factors which have been considered as being different in Spencer and Stern's experiment and these experiments seem unlikely to be the cause for the difference in the results. Since both Raychaudhuri's experiments and these experiments were performed with gamma rays, the difference of gamma rays vs. X rays in itself cannot be the reason for the difference between the results obtained by Spencer and Stern and those in this chapter. Furthermore, since Raychaudhuri worked at 8°C, a temperature effect seems unlikely, unless an unusually high stability of the genes at 18°C, declining both toward higher and lower temperatures, is assumed.

The aging effect, however, does not seem to be excluded, even though Raychaudhuri aged his sperm for 30 days, not 20 days as in this experiment. It is quite possible that the physiological aging process is disproportionally retarded at 8°C as compared with 18°C.

Finally, the dosages and intensities given must be compared. The lowest total dose given by Raychaudhuri was 8 times as high as that used in this experiment (400 vs. 50.5 r). The dose given per minute was 6 times as high (0.1 against 0.017 r/min), and the time of exposure was 50 per cent higher. The question is whether this difference can account for the difference observed in the experiments.

As mentioned previously, in the experiments reported in this chapter every sperm head received on the average 81 ion pairs in the course of the experiment, i.e., in 21 days. If the ionizations were distributed equally over the whole period, this would amount to one ion pair every 371 min, or about every 6 hr.

In Raychaudhuri's experiments a total of 400 r was given, corresponding to an average of 648 ion pairs per sperm head. One ion pair per sperm head would therefore be found every 67 min, or about once every hour.

Actually, these rate values are too high since the ionizations do not occur singly but in groups. Gamma rays form 1.5×10^5 ion pairs per centimeter of track,[22] or about 15 ion pairs per micron of track. Since the diameter of the head of Drosophila sperm is about 0.3 μ and its length is about 7 μ, the single ionization events will consist, on the average, of perhaps 5 to 10 ion pairs per sperm head. This would mean that, in Raychaudhuri's experiments, actually one ionization event (group of 5 to 10 ionizations, on the average) occurred every 5 hr per sperm head, or 72 to 144 single events per sperm head during the course of his experiment (Muller[23]). The corresponding values for the experiment reported here are that one group of 5 to 10 ionizations occurred per sperm head every 30 hr and that the average number of ion pair groups formed during the experiment per sperm head was between about 17 and 8.

In all these estimates a number of factors have been neglected, especially the higher degree of ionization due to the phosphorus contents of the chromatin. These calculations serve to show that the factor of 8 by which Raychaudhuri's experiments and these experiments differ involves a change in the order of magnitude of such a kind that the single events in the single sperm head, being only 17 on the average, would not be predictable with a high degree of certainty from statistical laws.

However, this difference cannot explain the small difference between experimentals and controls found in this series under the classical hit theory as elaborated by Timoféeff-Ressovsky, Zimmer, and Delbrück,[28] even under its broader formulation by Fano,[29] who included atom excitations among the primary events. This is due to the fact that it is a basic assumption of the theory that "the experimental law is valid beyond the present stage of investigation, i.e., is valid down to any low dosage and there is no dependence whatsoever on the time distribution of irradiation."[29]

Fano's analysis leaves open the possibility that interaction of "energy transfers" may occur. He mentions two kinds of interactions, "concentration effects," i.e., cooperative actions of two or more different adjacent ionizations or energy transfers, and quantum mechanical interference phenomena. In either case the independence of simple ionization events postulated by the classical hit theory would be abandoned. The reduction in difference between experimentals and controls found in this investigation would necessitate the assumption either that a threshold for the action of radiation exists or that two or more independent primary events are required for a mutation to occur. The dependence of a mutation on several primary events could again be imagined under different assumptions, such as interaction at the molecular level as proposed by Fano or at a cellular level as assumed in the recovery theory of physiological radiation effects.

6. CONCLUSIONS

The experiments reported in this chapter have shown that the difference between the mutation rates of irradiated and nonirradiated sperm is, with a high degree of probability, smaller if the irradiation is distributed over 21 days than if the same dose is given at once. A number of factors which may be responsible for this result have been discussed.

Although it seems unlikely that the wavelength of radiation, the temperature, and the chemical constitution of the containers have been the decisive factors, the aging of the sperm has not been excluded as a possible cause for this result. It must also be considered that a large number of small effects, such as the difference between glass and plastic containers, may have added up to obscure a really significant difference between experimentals and controls. Finally, it must be kept in mind that there are still 1.2 chances in 100 that Spencer and Stern's results and the results of this study have deviated from each other by chance only. This probability, although low, is not by any means an impossibility. On the other hand, if the relation between X-ray dosage and mutation rate is a linear one in general, then Spencer and Stern's result fits very closely the expectation. The probability of the data in this chapter being a chance deviation from such an expected value is lower than the one given above.

Before accepting the dependence of radiation effects on mutation rate at low doses on the time factor, it will be necessary to exclude all the factors discussed above which may have depressed the mutation rate in these experiments. If the result proves to be correct, it would necessitate a revision of the classical hit theory of induction of mutations. On the other hand, it would bring the production of mutations in line with a number of other effects of radiation, such as the production of chromosome rearrangements and the killing of bacteria and of Drosophila eggs.

From the practical point of view, the results presented open up the possibility that a tolerance dose for radiation may be found, as far as the production of mutations is concerned. This cannot be considered as proved, however, unless the other possibilities of explanation suggested in this chapter have been excluded.

Therefore no conclusion as to the practical application of these results seems to be permissible at this time.

7. SUMMARY

1. The rate of lethal sex-linked mutations in Drosophila exposed to gamma rays of 2.5 r/day for 21 days (total 52.5 r) has been determined.

2. In a total material of 108,215 chromosomes tested, no significant difference between experimentals and controls was found.

3. The difference between experimentals and controls found in this investigation is significantly ($P = 0.012$) different from that found by Spencer and Stern with the same dose of X rays given at once.

4. By comparison of the results of this study with those obtained by Spencer and Stern and by Raychaudhuri, it is concluded that it is unlikely that the relative inactivity of the irradiation is due to wavelength, temperature, or glass container.

5. As possible causes for the inactivity of irradiation in this experiment, different sensitivity of sperm at different stages of aging and dependence of induced mutation rate on a time factor at low dosages are being considered.

6. The latter explanation could form the basis of a tolerance dose for mutations. It cannot be accepted, however, unless the other possibilities of explanation are definitely excluded.

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Appendix

THE GENETIC EFFECTS OF LOW-INTENSITY IRRADIATION*

By Delta E. Uphoff and Curt Stern†

It has been shown by Spencer and Stern (Chap. 7) that irradiation by X rays at high intensity induces mutations in sperm of Drosophila melanogaster at dosages as low as 50 and 25 r and that the proportionality between dose and mutation frequency is maintained down to these low dosages. Earlier workers had established independence of induced mutation frequency from the intensity of irradiation at high and medium dosages. In contrast to these findings, Caspari and Stern (Chap. 15) obtained no significant difference in mutation rates between controls and experimentals, which had been subjected to a dose of 52.5 r in gamma rays administered continuously for 21 days at a rate of 2.5 r/day.

This unexpected result required further tests. After consideration of various factors, the following were regarded as possible causes for the apparent inactivity of irradiation in the experiment by Caspari and Stern: (1) low sensitivity to irradiation of aged sperm, (2) dependence of induced mutation frequency at low dosages on a time factor, and (3) errors of sampling which might have obscured a true difference between control and experimental rates. The first possibility was studied by administering 50 r in gamma rays continuously over 24 hr to sperm which had been aged previously for 20 days in the spermathecae of females.[30] In this experiment the intensity of irradiation was raised several times over that used by Raychaudhuri,[27] who had found typical intensity independence of mutation rates. Any deviation from the effect of irradiation expected for 50 r at medium intensity would thus be due to a specific sensitivity or insensitivity of the aged sperm. The second possibility, interference by a time factor, was tested by increasing the intensity of irradiation, and the total dosage, by a factor of 2, that is, by administering 100 r instead of about 50 r through continuous gamma irradiation over 21 days. The third possibility, chance, was checked by a repetition of the original experiment, that is, by giving once more 52.5 r in gamma rays over 21 days. Parallel with each experiment, the spontaneous mutation rate was determined in a set of controls.

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Table 1 — Mutation Rates for Sex-linked Lethals in Sperm of Drosophila melanogaster After Different Types of Treatment

Treatment	No. of controls	No. of experimentals	Mutation rate, %			Significance of difference	
			Controls	Experimentals	Difference	χ^2	P
50 r, 2.3 – 5 min exposure, not aged (Spencer and Stern)	73,901	31,560	0.0974	0.2440	0.1466	33.67	$<<0.01$
52.5 r,* 21 days exposure, aged (Caspari and Stern†)	56,252	51,963	0.2489	0.2848	0.0359	1.31	0.26
50 r,* 24 hr exposure after 20 days aging	44,601	46,232	0.1682	0.2834	0.1152	13.31	$<<0.01$
100 r, 21 days exposure, aged	22,958	31,562	0.2352	0.4658	0.2306	19.23	$<<0.01$
52.5 r,* 21 days exposure, aged	36,184	29,424	0.1765	0.2542	0.0777	4.67	0.03

*Geometric errors in the administration of the radiation are larger than the difference between the values of 52.5 and 50 r.

† The mutation rates obtained by Caspari and Stern have been adjusted to the slightly different scoring of lethals used in the investigations reported here.

The data, together with the earlier findings by Spencer and Stern (Chap. 7) and Caspari and Stern (Chap. 15) are summarized in Table 1. It is seen that all three new tests gave an increased frequency of sex-linked mutations in the treated sperm as opposed to the controls. The experimental rate observed by Caspari and Stern is statistically in good agreement with later determinations. The control rate of

0.2489 per cent found by Caspari and Stern is higher than any one of the later control rates. By itself, a rate of 0.2489 per cent for sperm aged over 21 days as compared to 0.0974 per cent for sperm not aged (Table 1, line 1) seemed in line with the degree of increase expected, according to the experience of other workers, after such aging. The new data on the control mutation rate in aged sperm suggest considerable variation of age accumulation of mutations in the Canton-Special stock on which all tests were carried out.

A comparison of the differences in mutation frequencies between experimentals and controls in the chronic experiments (Table 1, lines 2 to 5) with the difference in the acute experiment (Table 1, line 1) should take into account the fact that the difference in the latter experiment is somewhat larger than expected on the basis of all of Spencer and Stern's data. The maximum likelihood calculation yields about 0.002 per cent induced sex-linked mutations per roentgen, which corresponds to an expected difference for the acute 50-r experiment of only 0.1000 per cent. Viewing all experiments together, it appears that irradiation at low dosages, administered at low intensity, induces mutations in Drosophila sperm. There is no threshold below which radiation fails to induce mutations.

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PART III

FRACTIONATED DOSES OF FAST NEUTRONS

Chapter 16*

FAST-NEUTRON-IRRADIATION PROCEDURE

By Ellice McDonald

The cyclotron of the Biochemical Research Foundation, Newark, Del., which made its first run on Feb. 12, 1938, is the only cyclotron built for biochemical and biological studies. The initial purpose of its construction was the further study of the biological effects of radiation, with which the Biochemical Research Foundation had been concerned since 1932, using high-voltage X and gamma radiation. For this reason a full staff, composed of about 50 people, was assigned to perform the biochemical and biological studies. This staff included organic chemists, biochemists, physical chemists, cytologists, enzymologists, and bacteriologists, forming a well-qualified group for the study of chronic exposures of animals to neutrons when this was requested by the Manhattan District. This was a joint undertaking between the Biochemical Research Foundation, which did the major part of the work including the irradiation of the animals and the gross pathology, and the University of Rochester Manhattan Project, which did the histopathology and statistical analyses.

Since this joint study did not require the services of all the staff at the same time, the arrangement was such that available research capacity which was not required would be allowed to continue the radiation work which had been going on previously in the laboratories, provided that it did not interfere with the work required for the Manhattan District.

The study at the Biochemical Research Foundation was somewhat restricted because the joint research required repeated daily irradiation doses less often than was convenient for combining the work, but the plans of the laboratory were adapted to that limitation, and research into the acute effects of neutron radiation, particularly as applied to possible mechanism, was continued. The results of this work may be found elsewhere.[1]

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* Based on Report AECD-2595.

In the cyclotron constructed by the Biochemical Research Foundation in 1937 and 1938, many features which were then new were incorporated and have been described in the literature.[2] Fortunately, few mistakes were made in its construction. This was due in part to fortunate decisions and in great part to E. O. Lawrence of the University of California, who gave much good advice and received Biochemical Research Foundation personnel in his laboratories to gain experience and instruction. In addition, whenever he made improvements in cyclotron construction, he immediately sent blueprints and designs without waiting for these to be made available by publication.

The cyclotron of the Biochemical Research Foundation was not designed for the very high voltages needed in studies of nuclear physics since these voltages are not required for biological work. It originally operated regularly up to 10 μa of deuterons at 10.2 Mev on an external target and 40 to 50 μa on an internal target placed between the dees.

With an improved ion source the instrument delivered a current of 30 μa at 11 Mev on an external target, and, although it was possible, it did not seem necessary to go beyond this for biological work. During this study the operating voltage was less than this voltage.

The irradiation procedure has been described elsewhere,[1] and that which follows is largely a duplication of that description.

The cyclotron was used as the source of fast-neutron radiation. The neutrons were produced by deuteron bombardment of beryllium inside the cyclotron chamber. Location of the cyclotron and target is shown in Fig. 16.1. The numbered rectangles represent positions for which relative dosage values were determined.

To obtain simultaneous exposures of animals of high- and low-level dosage groups, cages were placed in different positions. Cages placed nearer the target received radiation dosage at a higher rate than those more distant. Cages 4, 5, and 6 each held 2 dogs, 4 rabbits, or 24 rats at one time. Cage 7 was considerably smaller, having a capacity of only 1 rabbit or 10 rats.

1. NEUTRON ENERGY

The deuteron beam energy was 9.5 Mev. The beam direction is indicated by the arrow in Fig. 16.1. The neutrons were produced by the reaction

$$Be^9 + H^2 \rightarrow B^{10} + {}_0n^1$$

The maximum neutron energy was 13.5 Mev; these neutrons were emitted from the target in the direction of the incident deuterons (arrow in Fig. 16.1). The main intensity of radiation emitted in this direction was due to neutrons of approximately 6-Mev energy. This was deduced from the energy determinations of Aebersold and Anslow[3] for a similar neutron beam and from comparison with the neutron spectrum reported by Staub and Stephens.[4]

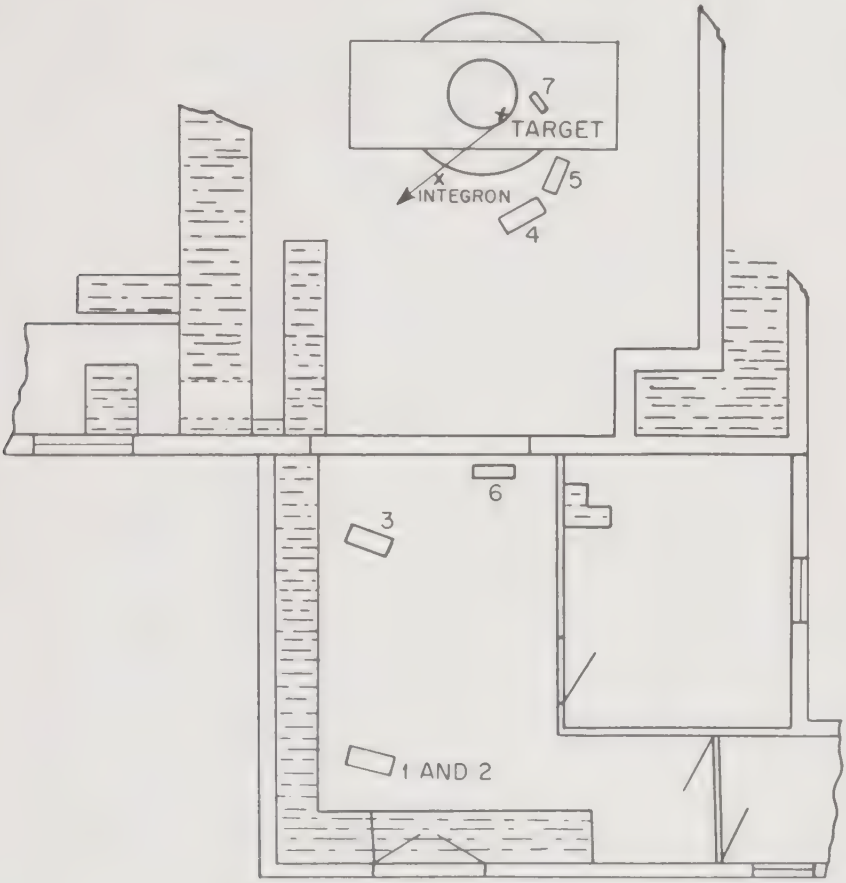

Fig. 16.1 — Plan of radiation area.

Positions 1 to 6 (Fig. 16.1) were as close to the line of maximum-energy neutrons as room dimensions permitted. For positions 1, 2, 3, 4, and 6 the deviation was not sufficient to decrease the energy of the peak neutron-radiation intensity. Position 7 was almost directly behind the target. The cage in this position received neutrons with a main intensity lower than 6 Mev but not lower than 2 Mev. It did not, however, receive any greater proportion of slow neutrons than the cage in position 4. Cage 5 received a main neutron-radiation intensity lower than that of cage 4 but higher than that of cage 7. The energy of the main-intensity neutrons was estimated as 3 Mev for position 7 and 5 Mev for position 5. Care was also taken to arrange the cages so that no cage was interposed between the beryllium target and any other cage.

Since positions 2 and 3 were near the water walls, they received thermal (very slow) neutron radiation formed by slowing down of fast neutrons in the water walls. These neutrons were removed by covering the water walls with cadmium absorbers.

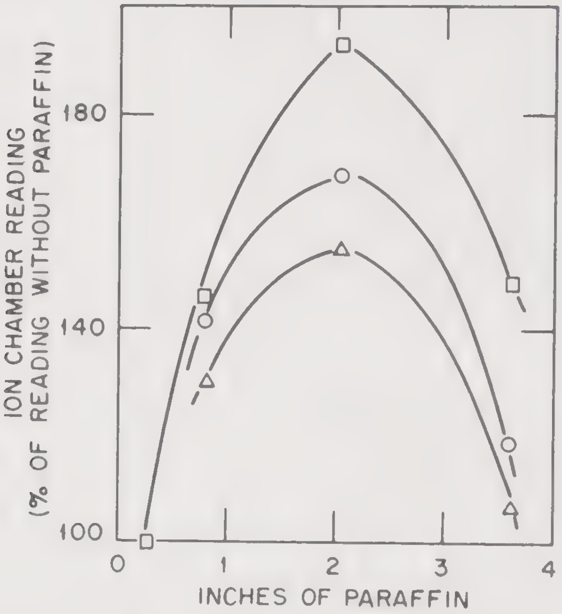

Fig. 16.2—Comparison of the neutron-intensity spectra in positions 4, 3, and 2. □, position 4. ○, position 3. △, position 2.

The neutron-intensity spectra in positions 2, 3, and 4 were compared by surrounding a 0.25-r Victoreen ionization chamber with successively thicker layers of paraffin and measuring the amounts of chamber discharge caused by equal neutron dosages emitted by the cyclotron. The results are shown graphically in Fig. 16.2.

Fast neutrons striking the paraffin are slowed and deflected so that they produce an increase of ions in the chamber. A peak intensity is reached when sufficient paraffin has been used to slow down all the fast neutrons. Further addition of paraffin cuts down the neutron intensity since the extra paraffin absorbs the slow neutrons. Hence the paraffin thickness at which maximum intensity occurs is greater if fast neutrons predominate in the radiation field.

Measurements made before the installation of the cadmium are shown in Fig. 16.3. Cage 2 has a maximum intensity point for a very thin paraffin absorber, indicating a high slow-neutron intensity point previous to installation of the cadmium shielding.

The curves of Fig. 16.2 are approximately identical. Position 4 was close to the target and presumably received most of its neutron-radiation intensity from 6-Mev neutrons. Hence, positions 2 and 3 received neutron radiation with a maximum intensity of approximately the same energy.

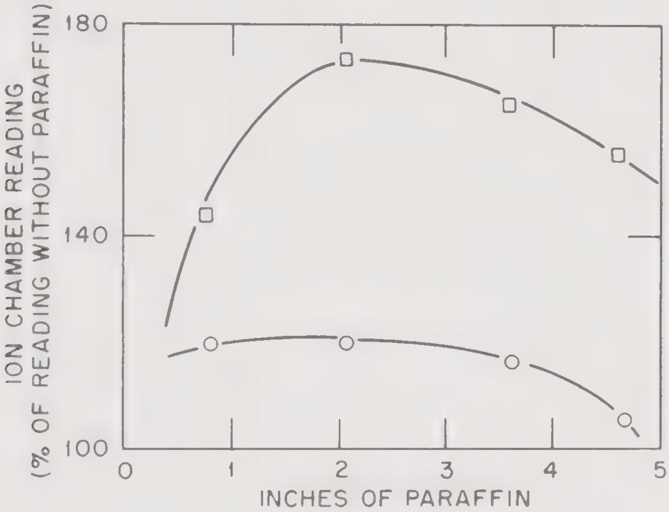

Fig. 16.3—Neutron intensity prior to installation of the cadmium shielding. □, position 4. ○, position 2.

2. NEUTRON-RADIATION INTENSITY

The method of intensity measurement followed that of Aebersold and Lawrence.[5] At the time of writing there was no well-defined standard of fast-neutron-radiation intensity. The method of Aebersold and Lawrence seemed the most practical, and, since this work was with a neutron spectrum similar to theirs, a comparison of results was easier. An important advantage in this method is that all radiation-intensity measurements are made with a Victoreen 100-r chamber. These chambers are commonly used in X-ray work and are readily available.

The Victoreen chamber gives its reading in roentgens (r). Following the practice of Aebersold and Lawrence, these readings are called "n" (neutron-radiation intensity) units. This was done for two reasons. The roentgen refers only to X (roentgen) radiation. The biological effect of an n unit of neutrons is different from that of the roentgen of X rays even though both give the same reading when measured with a Victoreen 100-r chamber.

The dosages were controlled by a Victoreen integron, an instrument which consists of an ionization chamber, amplifier, and meter. The meter indicates the chamber charge in roentgens and may be set at any dosage value. The reading decreases as the ionization chamber is exposed to radiation until the reading becomes zero. Thus, if the chamber is to receive 10 r, the meter is set at 10 r, and the chamber is exposed. When the chamber has received the 10 r, the meter reads zero and closes a relay which rings a bell and turns on a light. This relay is connected to the cyclotron oscillator in such a way that it turns off the oscillator and hence stops radiation when the exposure is completed and the meter reads zero.

Table 16.1 —Chamber Readings Corresponding to a 29.3-division Integron Discharge

Date	Total chamber readings, n	Total integron divisions
3/27/45	274.5	29.3 × 160
12/27/45	63.8	29.3 × 40
4/2/46	102.7	29.3 × 60
Total	441.0	29.3 × 260

It was impractical to mount the integron ion chamber in the cages; therefore it was placed in the position indicated in Fig. 16.1. As in the case of the cages, it was surrounded by 1 in. of lead. Four 100-r chambers were placed in cage 4, and their discharge was compared with the integron discharge reading. In successive trials the integron position was changed until a discharge of 29.3 divisions (full-scale reading is 30 divisions) corresponded to a mean dosage of 1.7 n in position 4. This relation of integron discharge to cage 4 was checked three times in the course of a year.

The 100-r-chamber readings corresponding to a 29.3-division discharge of the integron are given in Table 16.1. These measurements give a mean dosage for position 4 of 1.7 n for a 29.3-division discharge of the integron.

Dosages for positions 5, 6, and 7 were determined directly by placing four Victoreen 100-r ion chambers in the cages. The mean values obtained for different segments of the cages are recorded in Table 16.2 in terms of the dosage for position 4 obtained during the same exposure time.

Table 16.2 — Mean Values Obtained for Different Segments of the Cages

Cage No.	Ratio of dosage	Dosage for 29.3 integron divisions, n
4	100	1.7
5	63.2	1.07
6	12.6	0.12
7	395	6.72

3. PROTECTION OF PERSONNEL

The cyclotron was provided with both earth and water shielding and was located at some distance from the other laboratories. The main water walls were 3 ft thick, and the roof was covered with 2 ft of water. Depression of the cyclotron-room floor level placed the plane of the beam, and hence the plane of maximum radiation, just below the floor level of the main building. The long duration and high intensity of neutron radiation in this program made it necessary to evaluate all this protection. It was also essential that provisions be made against accidental entry into the radiation area.

A plan of the cyclotron room, animal house, and part of the main building is shown in Fig. 16.4. The neutron-radiation dosage in n units for normal daily operation (6 days per week) is shown by the numbers in the area where measurements were made. This dosage was exceeded during the last year of the program but did not average double the earlier dosage. Thus, doubling the dosage values given in the diagram gives the maximum average daily dosages at any of the points.

Point A (Fig. 16.4) represents the point of closest approach to the radiation area by cyclotron operators during normal operation. Point C is the limit of permitted approach during radiation. Point B is the limit of approach permitted in the animal house. Illuminated warning signs at points C and D automatically went on during cyclotron operation. An additional safety device was a photoelectric cell installed at

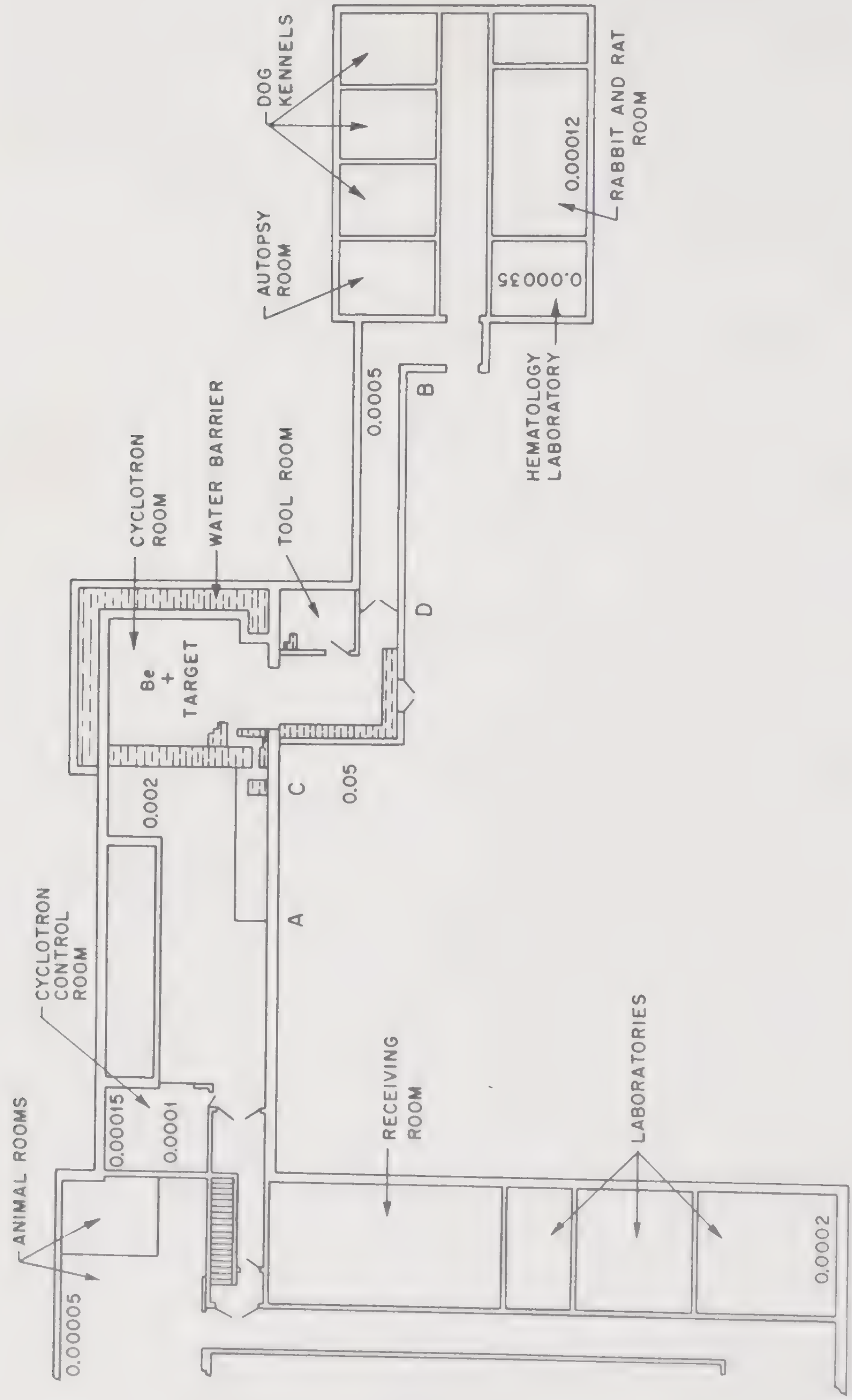

Fig. 16.4—Plan of the cyclotron room.

D which operated a bell signal in the control room so that the operator could stop irradiation if the cyclotron room was inadvertently entered from the animal house.

With reasonable care by the cyclotron operators, it was thus impossible for anyone connected with the radiation program to receive an accumulative dosage of more than 0.001 n/day. Actual dosages received by personnel were probably a fraction of this. Blood examinations were made periodically on all operators and attendants who might be exposed to radiations.

Additional radiation monitoring was achieved by film badges worn by personnel throughout the program. These consisted of dental films set in brass frames. A portion of the film was covered with a cadmium shield to absorb neutrons; this area was used to monitor the gamma radiation only. These badges were checked weekly and made possible the detection of any accidental entry into the radiation area. During the program there was no evidence of any badge being exposed to detectable amounts of radiation while worn by personnel.

4. EXPERIMENTAL ANIMALS

In these experiments 340 animals were used and consisted of the following: 250 rats of the Wistar strain (one-half were males and one-half were females), 60 male rabbits of the New Zealand White breed, and 30 dogs of mixed breed, predominantly terrier. The animals were divided into groups as shown in Table 16.3.

Table 16.3 — Grouping of Experimental Animals

	Number of rats		Number of rabbits		Number of dogs		
Group No.	Males	Females	Males	Females	Males	Females	Dosage, n/day
00	25	25	20	0	3	7	0.000
01	25	25	10	0	3	2	0.012
02	25	25	10	0	3	2	0.060
03	25	25	10	0	3	2	0.110
04	25	25	10	0	3	2	1.700

4.1 Preparation of Animals for Experimental Use. (a) Rats. Vigorous and healthy rats were selected. An attempt was made to eliminate all those which had any respiratory infection. However, because of the difficulty involved in doing this, there were some rats with slight sniffles in the groups when the experiments were begun. The rats averaged approximately 225 g at the beginning of the experiment.

(b) Rabbits. The rabbits were obtained approximately 3 months before the initiation of irradiation, at which time they averaged 3 months of age. Fecal examinations showed that nearly 100 per cent of the animals were infected with coccidiosis. Daily sterilization of cages and of food and water containers, along with other hygienic measures, improved the condition of the colony so that, as determined by fecal examination, the infestation had been eliminated.

(c) Dogs. The dogs were acquired from dealers in the surrounding area approximately 3 months prior to the beginning of the irradiation experiments. The majority were about 1 year of age with some ranging up to 3 years. On admittance, all dogs were given a prophylactic dose of Anti-Canine-Distemper Serum (Pitman-Moore Co.). Fecal examinations were made, and after the usual preparatory period the dogs were medicated with arecoline and santonin for cestodes and with tetrachloroethylene and n-butyl chloride for intestinal nematodes. Fecal examinations of all dogs were made weekly until three successive examinations proved negative. The dogs were all given canine distemper vaccine (Virogen) in three doses at 14-day intervals and were vaccinated with (phenolized suspension) rabies vaccine (Fort Dodge Serum Co.). Blood examinations for Dirofilaria immitis were all negative. Any pneumonic or intestinal condition that appeared during the preparatory period was treated according to the nature of the ailment. The dogs were placed, from the time of their admittance until 2 weeks prior to irradiation, on a diet consisting of Purina Fox Chow Meal, supplemented with ground horse meat and beef with a liberal allowance of Co-Fer yeast (Pitman-Moore Co.). When the state of an animal required vitamin therapy, additional vitamins were given. Before irradiation was begun, the dogs were given a final physical examination; their ears were cleaned, their toenails were clipped, and tartar was removed from their teeth.

4.2 Animal Care and Irradiation Procedure. All animals were housed in a building constructed of concrete-cinder blocks with a concrete floor.

The temperature in the several rooms was thermostatically controlled but varied considerably owing to weather changes, light construction of the building, and lack of precision of the thermostatic control. The temperature of the room in which the rats and rabbits were kept together varied from 68 to 78°C except in hot summer weather, when higher temperatures were reached.

The rats were kept in pairs according to sex in all-wire cages. They were maintained on Fox Chow containing meat meal, with a supplement of Fox Blox over the week end. Water was available at all times. Weighings and clinical examinations were made usually at 1-week intervals and occasionally at 2-week intervals.

When the rats were to be irradiated, each was placed into a compartment of a copper box (Fig. 16.5). Each compartment was 8 by 2½ by 2¾ in., which kept the animal oriented with one side toward the source of radiation. It was possible for some of the smaller rats to reverse their position in the compartment.

Each of the copper boxes contained 12 compartments. Provision for the ventilation of each compartment was made by three ⁵⁄₁₆-in. holes in the back and 33 holes of the same size in the front lid.

The copper boxes containing the rats were carried to the radiation room and placed into the copper-lined lead boxes for exposure. Each lead box accommodated two of the rat-holding boxes; therefore 24 rats could be irradiated at a time in each lead box. After irradiation the rats were returned to their cages.

The rabbits were kept in the same room with the rats in individual wire cages and were maintained on equal parts of whole oats and Purina Rabbit Chow. Whenever sore hocks occurred, they were treated with a salve of 10 per cent iodine in lanolin. A masonite board was placed in each cage as a prophylactic measure. Otodectic mite infection was treated with a standard oil treatment. The rabbits were weighed and clinical examinations were made usually at 1-week intervals and occasionally at 2-week intervals.

When the rabbits were to be irradiated, they were placed into individual copper boxes (Fig. 16.6), approximately 5 by 9 by 12 in., which were transported to the radiation room and placed into the copper-lined lead boxes for exposure, after which they were returned to their cages. The position of each rabbit was reversed on alternate days so that alternately one side and then the other was exposed toward the source of radiation.

The dogs were kept in individual stalls, approximately 2½ by 3½ by 4 ft. The floor was concrete, but each cage had a wooden floor in a box raised several inches from the concrete floor. The wooden box, covering approximately one-half the entire floor space of a cage, served as sleeping quarters or for occupancy at any time.

When the dogs were to be irradiated, they were taken to the irradiation room and placed directly into the copper-lined lead boxes for exposure, after which they were returned to their stalls.

The dogs were weighed and examined clinically usually once a week and occasionally once in 2 weeks.

During the experiment the dogs were maintained on a diet of Friskies. They were fed once a day, 6 days a week, and fasted on Sunday. Water was available at all times.

The lead boxes in which the animals were placed for irradiation (Figs. 16.7 and 16.8) were constructed of lead sheets 1 in. in thickness and lined with copper sheeting. The front section or lid of the

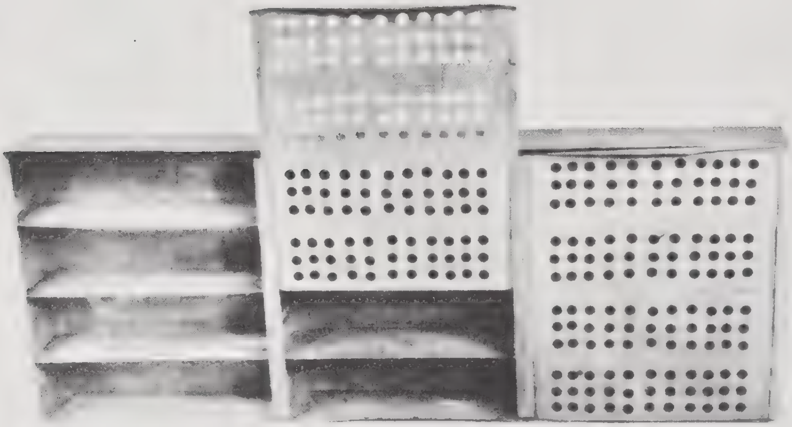

Fig. 16.5—Copper box for holding rats during irradiation.

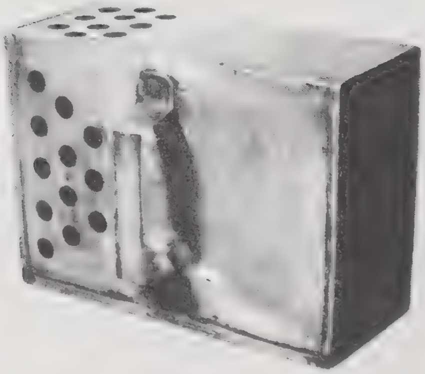

Fig. 16.6—Copper box for holding rabbits during irradiation.

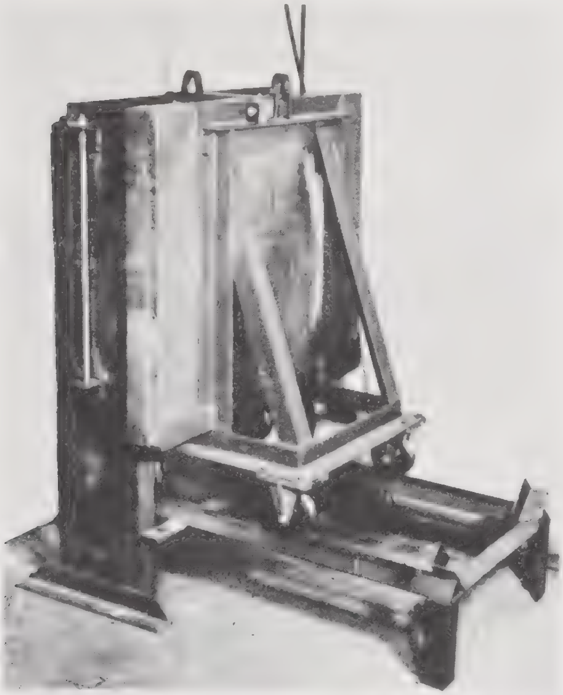

Fig. 16.7 — Lead box to contain animals during irradiation (closed).

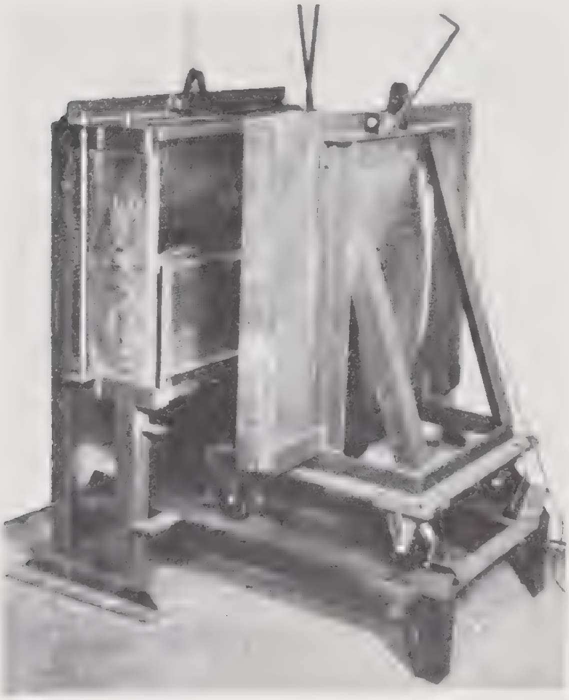

Fig. 16.8 — Lead box to contain animals during irradiation (open).

box was made slightly larger than the outside dimensions of the box and was not allowed to close completely so that, when closed, there was a space between the box and lid for air circulation. The outside of the lid overlapping the box prevented unfiltered radiation from entering the box.

The lead boxes were ventilated by forcing air through the back. This was done by the installation of a ventilator into each of the two compartments of a box. Air entering the ventilator under slight pressure made its exit through small holes distributed in the side of the ventilator next to the animal. The circulation of air was not rapid enough to cause drafts. Figure 16.9 shows the cyclotron and the preparation of cage 4 for an experiment.

The animals were irradiated 6 days per week for 1 year; irradiation was omitted on Christmas Day. There was one occasion when a single day of irradiation was omitted, but this was performed on the following Sunday. On one other occasion 2 days of irradiation were not made according to schedule, but these 2 days were made up on the succeeding two Sundays.

The approximate length of time required to administer the daily treatment for each dose level was, for group 01, 1 to 2 min and, for groups 02, 03, and 04, 5 to 6 min. The animals of the control or 00 group were placed in the boxes for the average length of time required for the other groups to be irradiated.

With few exceptions all animals that died were autopsied within ½ hr of death. Those which survived the 1-year period were killed and autopsied immediately at intervals during the following 3 months.

ACKNOWLEDGMENTS

The designs for the cages were worked out in the laboratories largely by the late H. M. Terrill and by Claude Ludwig, the instrument maker, who made the cages and offered many good suggestions. Dosage measurement was studied in conjunction with the Rochester Manhattan Project physicists. The animal care, in a report of work such as this, is apt to be obscured by the irradiation description, but it is equally important because, if standards of normality of animals are not maintained as a background and variables are not eliminated, the most exact measurement of the physical force is of little value. The chief animal caretaker, James Duffy, had this responsibility and made several very interesting new suggestions. Col. Dewey M. Stowers, local representative of the Manhattan Engineering District, was persevering in his efforts to aid in the progress of the Project and was instrumental in obtaining the help of the Manhattan Engineer-

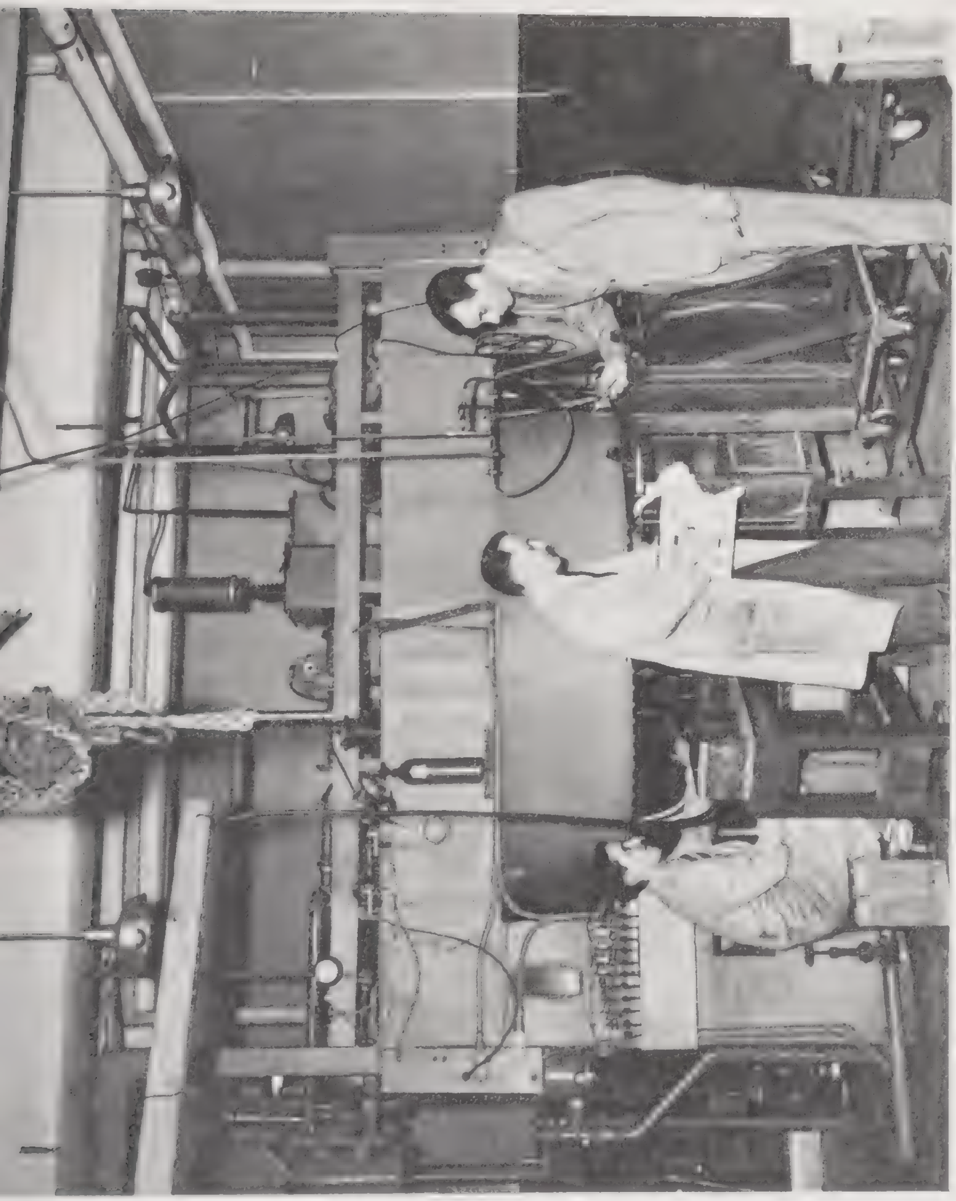

Fig. 16.9—Cyclotron and cage arrangement.

ing District in this work. Without his help and understanding, this part of the Project would have lagged. Indeed, there were many persons who devoted all their energies to the completion of the work; without their assistance the Project would have been seriously hampered.

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Chapter 17*

CLINICAL, PATHOLOGICAL, AND HEMATOLOGICAL EFFECTS OF CHRONIC NEUTRON RADIATION

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1. INTRODUCTION

Studies of the effects of chronic neutron irradiation of rats, rabbits, and dogs were carried out for the U. S. Army Engineer Corps, Manhattan District, as a joint undertaking of the Biochemical Research Foundation at Newark, Del., and the Manhattan Project at the University of Rochester. The work was divided in such a manner that the neutron irradiation as well as the clinical, gross pathological, and hematological studies were carried out at the Biochemical Research Foundation, whereas the microscopic pathology and statistical analysis of hematological findings were done at the University of Rochester. In a few instances, certain minor differences may be noted between the interpretations of the microscopic and gross pathologies. These differences reflect a problem inherent in the division of a single project between two physically separated groups.

2. METHODS

2.1 General. Extensive details of animal selection and care and radiation techniques are presented in Chap. 16. The general plan of the experiment was to expose rats, rabbits, and dogs to a neutron beam from the Delaware cyclotron daily, 6 days a week, for 1 year. Table 17.1 summarizes the general protocol and indicates the group numbers which will be used throughout this chapter in referring to the various dosage groups.

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*Based on Report UR-92, Jan. 16, 1950, and edited by M. B. Ingram and W. B. Mason.

2.2 Clinical Observations. The clinical data which were collected pertain to the general condition of the animals, weight changes, mortality, incidence of cataracts, changes in character and color of the coats, and incidence of grossly apparent tumors. For convenience, in some instances, the gross post-mortem findings are included under clinical observations.

Table 17.1—Grouping of Experimental Animals

Group	Number of rats		Number of rabbits		Number of dogs		Dosage, n/day
	Males	Females	Males	Females	Males	Females	
00	25	25	20	0	3	7	0
01	25	25	10	0	3	2	0.012
02	25	25	10	0	3	2	0.06
03	25	25	10	0	3	2	0.11
04	25	25	10	0	3	2	1.7

2.3 Pathology. With few exceptions, all animals that died were autopsied within ½ hr of death. Those which survived the 1-year experimental period were killed and autopsied at intervals during the following 3 months.

Tissue samples fixed in 10 per cent neutral formalin were submitted to the Pathology Division in Rochester for microscopic examination. The following organs were sampled in most instances: heart, lung, liver, kidney, spleen, lymph nodes (dogs only), bowel, stomach, pancreas, thyroid, adrenal, gonad, skin, and vertebral and femoral bone marrow. These tissue samples were embedded in paraffin; 7-μ-thick sections were cut and stained with hematoxylin and eosin. Bone-marrow smears taken from the mid-diaphysis of the femur were stained with Wright's stain and used in making differential counts of marrow elements.

2.4 Hematology. Hemograms were determined at Delaware at intervals which are recorded in Table 17.2. At the completion of the experiment the data were sent to Rochester for interpretation and statistical analysis.

In all the acute neutron experiments, red and white blood cell counts, reticulocyte count, platelet count, hemoglobin determination, differential white blood cell count, and an appraisal of poikilocytosis, anisocytosis, basophilia, and stippling constituted a complete blood count. The counts were all made according to standard procedures. Hemoglobin determinations were made on a Klett-Summerson colorimeter. Differential blood counts were based on the classification of

100 leucocytes on a Wright's stained blood smear. Reticulocyte counts were done on Wright's stained cover-slip smears which were made by using one plain cover slip and one cover slip on which a drop of brilliant cresyl blue had been allowed to dry. The number of reticulocytes found in 1000 cells was determined, and the incidence was recorded in percentages.

Table 17.2 — Frequency of Hemograms

Species	Group	Dosage, n/day	Frequency of blood counts
Rats, rabbits, and dogs	00	Controls	Weekly
	01	0.012	Monthly
	02	0.060	Monthly
	03	0.110	Monthly
	04	1.700	Weekly

Dogs were bled from the jugular vein. Platelet counts and cover-slip blood smears for Wright's staining and reticulocyte counts were made directly from the blood flowing from the needle. All other counts were made on oxalated blood. Rats were bled by cutting approximately ⅛ in. off the end of the tail. All counts were made on the freely flowing blood.

3. RESULTS

3.1 Clinical Observations Pertaining to Rats. (a) General Conditions. There was no clinical change in groups 00, 01, 02, and 03 that could be attributed to the effects of radiation. Group 04 rats, however, showed marked changes. During the first 25 weeks, rats of this group barely maintained their initial weights. Beginning at the twenty-fifth week they lost weight progressively until death. Early in the experiment, rough coats characterized the group. Activity was reduced and sluggish, and the animals failed to keep themselves clean; their tails and feet became encrusted with dirt. During the later stages of their lives they sat with arched backs in their feed cups, apparently asleep most of the time. Food intake was greatly reduced, and extreme emaciation resulted.

(b) Alopecia and Thinning of Hair. Thin hair and alopecia appeared in approximately equal numbers in the various groups but appeared somewhat earlier in the more heavily irradiated groups than in the control group (Table 17.3).

(c) Weight. Figure 17.1 shows in graphic form the weight changes of the rats of the various groups. It may be seen that the weight increase of groups 00, 01, 02, and 03 paralleled each other closely, whereas the rats of group 04 barely maintained their initial weight during the first half of the experimental period, after which the average weight declined rapidly until all animals were dead.

Table 17.3 — Incidence of Thin Hair and Alopecia in Rats*

Day of experiment	Number of treatments	Group 00		Group 01		Group 02		Group 03		Group 04	
		T	P	T	P	T	P	T	P	T	P
1	1										
33	28										
61	52										
75	54										
96	82										
117	100										
143	122										
173	148										
201	172										1
215	184										
229	196										3
243	208										1
257	220							1	3		
263	225										
285	244								1	3	3
299	256	1				6		3			
313	267		1		3	2	2				
327	279	2	2	1	1	3		3	3		
341	291	8	1	2	1	6	1	6	2		
355	303	5	1	2	1	2	1	11	3		
362	309	2	2	6	2	4	2	4	3		
369	312	1		2	1	3			2		

*T, thin hair; P, alopecia.

(d) Mortality. All rats of group 04 were dead at the end of 223 treatments. In contrast to this, the following numbers in the other groups were still living at the end of the year of 312 radiation treatments: group 00, 68 per cent; group 01, 64 per cent; group 02, 68 per cent; and group 03, 62 per cent.

Table 17.4 shows the mortality data for rats. Statistically, there was no difference in the rate of mortality in groups 00, 01, and 02. Figure 17.2 shows that the rate of mortality in group 04 was greatly increased over that of the control animals. Figure 17.2 shows also a slightly more rapid death rate in group 03 than in groups 00, 01, and 02. This is of questionable significance. However, if the mortality data for these groups are divided into three periods in relation to the number of treatments (Table 17.5), it is seen that there was a 100 per cent increase in the mortality rate of group 03 over that of the other groups in the period from 200 to 312 treatments. It seems possible that the cumulative effect of the first 200 treatments exerted an influence during the last portion of the treatments.

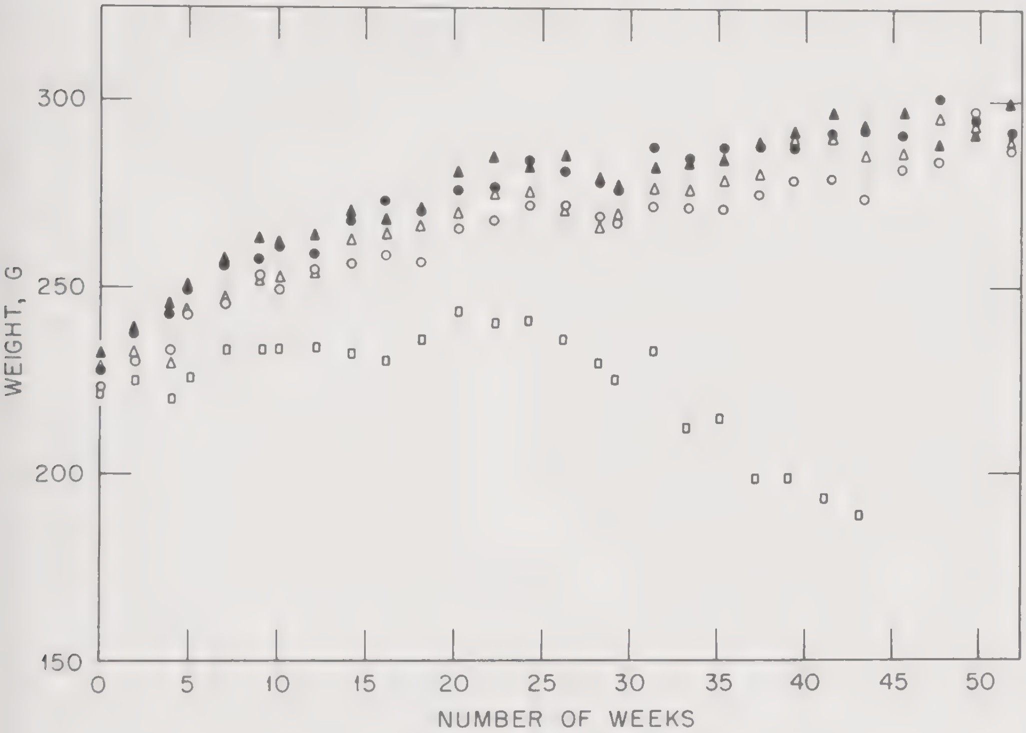

Fig. 17.1—Weights of rats during the period of daily irradiation with neutrons. ○, group 00. ●, group 01. △, group 02. ▲, group 03. □, group 04.

(e) Incidence of Cataracts. A peculiar type of cataract, involving primarily the eyes of group 04 rats of both sexes, occurred after about 200 treatments of irradiation with neutrons (Table 17.6). At this time there were 26 survivors of the original 50 rats. Two females were discovered, after 202 treatments (dose, 343.3 n), to have marked lenticular opacities involving the entire lens. These animals were blind. The remaining rats were carefully examined for incipient cataracts, but it was not until the following week that others of this group were diagnosed as having cataracts. At this period, after 208 treatments, 9 of the remaining 20 rats showed the gross clinical manifestations of cataract. The number of cataracts in relation to

Table 17.4 — Mortality Data for Rats

Experimental group*	Animal No.	Days in expt. to death date	No. of treatments	Total dose, n	Percentage of group surviving
00	42	55	48	0	98
	34	79	67	0	96
	12	113	96	0	94
	11	113	97	0	92
	01	116	99	0	90
	07	159	136	0	88
	23	173	148	0	86
	38	179	154	0	84
	30	195	168	0	82
	06	204	174	0	80
	36	222	191	0	78
	03	283	243	0	76
	32	288	246	0	74
	17	314	269	0	72
	37	333	285	0	70
	14	362	310	0	68
01	10	60	52	0.625	98
	18	72	61	0.732	96
	50	82	71	0.852	94
	20	94	81	0.972	92
	09	109	94	1.128	90
	04	132	101	1.212	88
	32	163	126	1.512	86
	40	188	162	1.944	84
	17	200	171	2.052	82
	44	293	252	3.024	80
	48	312	267	3.204	78
	14	316	269	3.228	76
	39	321	274	3.288	74
	01	322	275	3.300	72
	25	340	290	3.480	70
	46	357	305	3.660	68
	19	359	307	3.684	66
	08	363	311	3.732	64
02	02	11	10	0.60	98
	19	76	66	3.96	96
	24	84	72	4.32	94
	14	136	117	7.02	92
	18	146	124	7.44	90
	26	186	160	9.60	88
	25	188	162	9.72	86
	47	209	180	10.80	84
	49	248	212	12.72	82
	13	267	229	13.74	80
	35	281	240	14.40	78
	22	295	252	15.20	76
	06	309	263	15.78	74
	01	325	278	16.68	72
	08	330	281	16.86	70
	16	348	297	17.82	68
03	49	186	160	17.60	98
	17	189	162	17.82	96
	10	232	199	21.89	94
	34	237	204	22.44	92
	21	238	204	22.44	90
	15	253	217	23.87	88
	45	255	219	24.09	86
	47	261	223	24.53	84
	12	261	223	24.53	82
	26	265	227	24.97	80
03	38	284	243	26.73	78
	08	292	251	27.61	76
	06	307	262	28.82	74
	04	333	284	31.24	72
	28	344	294	32.34	70
	48	344	294	32.34	68
	41	352	301	33.11	66
	33	353	302	33.22	64
	35	356	305	33.55	62
04	02	29	24	40.8	98
	46	31	27	45.9	96
	21	90	77	130.9	94
	03	95	82	139.4	92
	24	132	113	192.1	90
	42	146	123	209.1	88
	36	163	140	238.0	86
	28	186	160	272.0	84
	09	196	168	285.6	82
	10	202	173	294.1	80
	27	204	174	295.8	78
	25	205	176	299.2	76
	18	208	178	302.6	74
	11	211	180	306.0	72
	48	212	181	307.7	70
	26	213	183	311.1	68
	16	216	185	314.5	66
	19	219	188	319.6	64
	33	219	188	319.6	62
	17	224	192	326.4	60
	05	228	195	331.5	58
	37	228	195	331.5	56
	30	235	201	341.7	54
	31	235	202	343.4	52
	23	236	203	345.1	50
	29	238	204	346.8	48
	13	239	204	346.8	46
	45	239	205	348.5	44
	32	241	207	351.9	42
	20	242	207	351.9	40
	34	249	214	363.8	38
	12	251	216	367.2	36
	22	252	216	367.2	34
	50	252	216	367.2	32
	01	253	216	367.2	30
	07	256	220	374.0	28
	04	257	222	377.4	26
	39	259	222	377.4	24
	15	261	223	379.1	22
	35	261	223	379.1	20
	14	262	224	380.8	18
	41	263	225	382.5	16
	08	263	226	384.2	14
	49	265	228	387.6	12
	38	269	230	391.0	10
	44	270	232	394.4	8
	47	281	241	409.7	6
	40	292	250	425.0	4
	06	295	253	430.1	2
	43	300	283	481.1	0

* Each group was composed of 50 rats.

the number of surviving rats of this group increased, so that, after 237 treatments, the four remaining rats all had cataracts.

Of the total number of rats initially in the group, 21, or 42 per cent, ultimately had cataracts; 9 of these were males, and 12 were females. The opacities occurred relatively late in the experiment and might have had a higher incidence if the number of animals surviving after 200 treatments had been greater. It is worth mentioning at this point that the incidence of cataract formation occurred in the period during which the number of survivors was decreasing at a rapid rate as compared to other groups.

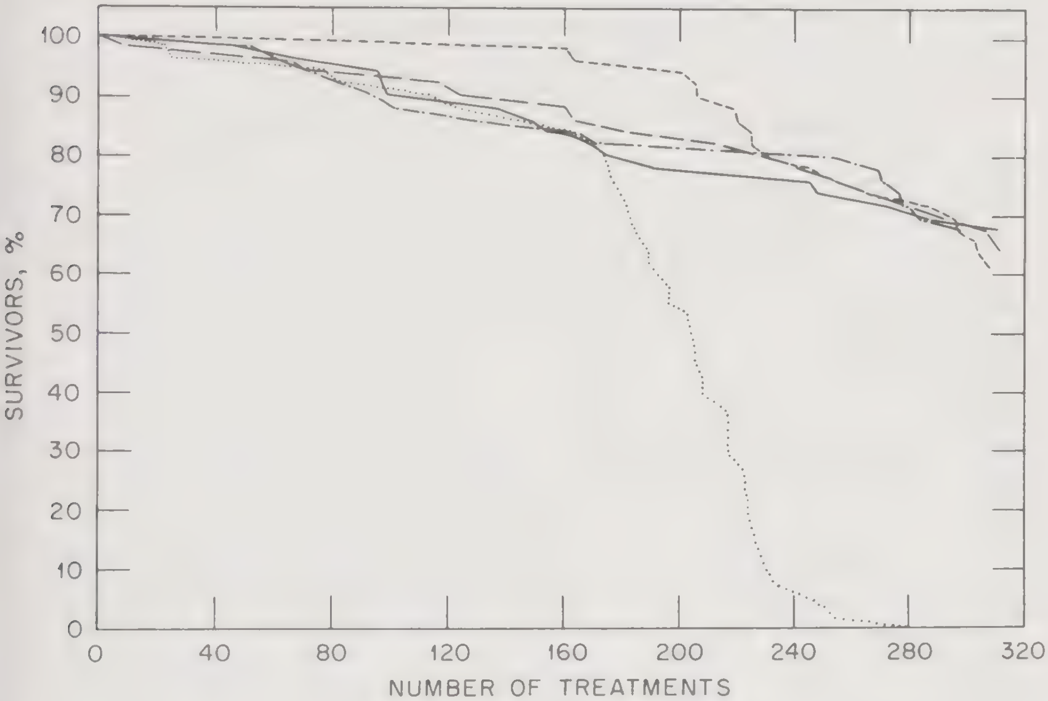

Fig. 17.2—Mortality of rats during the period of daily irradiation with neutrons. ——, group 00. —-, group 01. — —, group 02. ----, group 03. ·····, group 04.

It was difficult to study the sequence of changes that occurred in the eyes of these rats. Examinations were made with the ophthalmoscope after installation of 0.01 per cent atropine sulfate into the conjunctival sac. Some details were observable, but extensive studies were not carried out. The nature of these cataracts was obscure and of several different types. All types progressed to a complete milky opacity of the lens with consequent blindness of the animal. The first sign in some was a small dark opacity on the center of the lens. Later this dark center became surrounded by a diffuse cloudiness which progressed to complete opacity in those animals which survived for some time. In other cases the opacity appeared to be of a granular nature. In the early stages the eye ground could be seen, but later the lens became too opaque for the eye-ground observations. In one case, animal 47, there was centrally located a star-shaped clear zone through which the eye ground could be observed, but the radiating lines to or from the periphery were quite opaque. Later this animal showed a diffuse cataract with fine blood vessels extending over the surface of the lens. Only rat 35 showed any pathological changes of the eye other than cataract. In this case the anterior chamber of the eye became very opaque and of a milky nature; thus it was impossible to examine the deeper structures.

Table 17.5 — Mortality of Rats at Various Periods of Treatment

Group	Number of treatments 0–100	100–200	200–312
00	5	6	5
01	5	4	9
02	3	5	8
03	0	3	16
04	4	18	28

Table 17.6 — Cataracts in Surviving Group 04 Rats

Date	Number of treatments	Dose, n	Number of living animals with cataracts	Number of survivors	Percentage of animals having cataracts
10/18/45	202	343.3	2	26	7.6
10/25/45	208	353.6	9	20	45.0
11/1/45	214	563.8	12	19	63.0
11/8/45	220	374.0	13	14	93.0
11/28/45	237	402.9	4	4	100.0

(f) Incidence of Grossly Apparent Tumors. The word "tumor" is used here to mean a growth, not inflammatory in character, arising from preexisting tissue. No distinction has been made between benign and malignant tumors since this chapter is based on clinical and gross pathological examinations. It should be noted also that very small tumors may have been overlooked and that microscopic examination of sections of the tissues may reveal tumors not included in this tabulation.

Table 17.7 shows the incidence of tumors in the various groups as found by clinical examination and autopsy. A summary of the number of tumors and their location in the various groups of rats and the percentage of rats having internal and external tumors are given in Table 17.8.

In group 00, the control group, no mammary tumors were found, in contrast to six tumors found in group 03. This is considered to be suggestive evidence only of an effect from the irradiation. Other than this, no possible significance is attached to the occurrence of the other external tumors.

The incidence of internal tumors was much greater in groups 01, 02, and 03 than in group 00. The majority of these tumors apparently originated in the lymph nodes at the caecum. Caecitis in several strains of rats has been described.[1] The preponderance of the internal tumors observed in these animals gave the impression that they may have originated from the enlarged lymph nodes associated with caecitis. These tumors were usually multiple, often very numerous, and varied from pin-point size to 1 in. in diameter. In some of the rats the tumors occupied most of the abdominal cavity, and in some cases the chain of tumors extended into the thoracic cavity. The much higher incidence and greater severity of tumor growth in the radiated groups of rats over the incidence in the control group suggest that the radiation may have been the inciting agent. However, the radiation may have only hastened the appearance of naturally occurring tumors.

3.2 Pathological Findings in Rats. Because it was considered advisable to allow some of the rats to survive beyond the period of treatment for studies other than histopathology and because deaths occurred in all groups during the treatment period, it has been necessary to divide the rats in the control group and in each of the experimental groups except group 04 into several subgroups, using the time and manner of death as a basis for this subgrouping. The number of animals in each category is shown in Table 17.9.

The largest of these categories and, from the standpoint of histopathology, the most important was that made up of the animals that died during the exposure period. The abnormalities observed in these rats are tabulated in Tables 17.10 to 17.14. Four general types of abnormality were encountered at all four dosage levels and in the control group: (1) spontaneous infectious diseases, (2) changes characterized by alterations (for the most part, atrophic in nature) in the normal cellularity of hematopoietic organs and gonads, (3) hemorrhagic manifestations, and (4) neoplasms.

Table 17.7—Incidence of Tumors in Rats

			Tumor data			Mortality data			
Experimental group	Dose, n/day	Animal No. and sex	Days in expt. until tumor was observed	Number of treatments	Total dose, n	Days in expt. until death date	Number of treatments	Total dose, n	Location and remarks
00	Control	03, M	284	243	0	284	243	0	Caecum; multiple nodules
		17, M	299	256	0	315	269	0	Thymus, abdomen; multiple nodules
		29, F	395	312	0	395	312	0	Mammary; multiple nodules
		48, F	366	312	0	366	312	0	Ear; single nodule at ear punch
		49, F	457	312	0	457	312	0	Thymus; thymus or lymphatic
01	0.012	11, M	379	312	3.74	379	312	3.74	Caecum; large lymphatic tumors
		17, M	194	166	1.99	201	171	2.05	Caecum-omentum; nodules
		28, F	166	142	1.70	367	312	3.74	Caecum, axilla, inguinal; lymphatic mammary?
		32, F	145	122	1.46	150	126	1.51	Peritoneal cavity, thymus; multiple nodules
		34, F	145	122	1.46	367	312	3.74	Caecum; large single
		44, F	294	252	3.02	294	352	3.02	Caecum; 2 tumors ½ in. dia.
		05, M	416	312	3.74	416	312	3.74	Omentum; multiple nodules
		06, M	397	312	3.74	397	312	3.74	Caecum, ileum, etc.; large, lobulated
		12, M	395	312	3.74	395	312	3.74	Caecum; viscous, lobulated
		27, F	455	312	3.74	455	312	3.74	Caecal area; cystic

Table 17.7—(Continued)

Experimental group	Dose, n/day	Animal No. and sex	Tumor data			Mortality data			Location and remarks
			Days in expt. until tumor was observed	Number of treatments	Total dose, n	Days in expt. until death date	Number of treatments	Total dose, n	
		43, F	413	312	3.74	455	312	3.74	Ovary; cystic, lobulated
		04, M	26	22	0.26	119	101	1.21	Front-leg axilla; large, solid
02	0.06	01, M	326	278	16.68	326	278	16.68	Thymus, caecum, etc.; multiple nodules
		06, M	310	263	15.78	310	263	15.78	Caecum mesentery; nodules
		13, M	215	184	11.04	268	229	13.74	Caecum; large single
		17, M	367	312	18.72	367	312	18.72	Caecum; large single
		20, M	392	312	18.72	392	312	18.72	Pancreas; multiple, nodular
		22, M	296	252	15.12	296	252	15.12	Caecum, etc., pancreas; multiple nodules
		36, F	368	312	18.72	368	312	18.72	Mammary
		49, F	249	212	12.72	249	212	12.72	Caecum, etc.
		50, F	396	312	18.72	396	312	18.72	Mammary
03	0.11	08, M	293	281	30.91	293	281	30.91	Mesentery; multiple, nodular
		10, M	233	199	21.89	233	199	21.89	Caecum, etc.; multiple, nodular
		16, M	370	312	34.30	370	312	34.30	Mesentery; chain of small tumors
		17, M	190	162	17.82	190	162	17.82	Caecum; several large nodes
		18, M	370	312	34.30	370	312	34.30	Caecum; small, cystic
		23, M	457	312	34.30	457	312	34.30	Caecum; multiple

Table 17.7 — (Continued)

			Tumor data			Mortality data			
Experimental group	Dose, n/day	Animal No. and sex	Days in expt. until tumor was observed	Number of treatments	Total dose, n	Days in expt. until death date	Number of treatments	Total dose, n	Location and remarks
		24, M	397	312	34.30	397	312	34.30	Caecum; multiple
		26, F	266	227	24.97	266	227	24.97	Caecum, etc.; multiple, large
		27, F	370	312	34.30	370	312	34.30	Caecum and neck regions; multiple
		29, F	397	312	34.30	397	312	34.30	Mammary region
		34, F	238	204	22.44	238	204	22.44	Mesentery; nodular
		32, F	414	312	34.30	426	312	34.30	Neck; nodular
		45, F	256	219	24.09	256	219	24.09	Caecum; large
		46, F	370	312	34.30	370	312	34.30	Caecum, mesentery; multiple nodules
		49, F	166	142	15.62	187	160	17.60	Caecum; large single
		06, M	308	262	28.82	308	262	28.82	Leg; metastases to lung
		12, M	257	220	24.20	262	223	24.53	Mammary
		35, F	215	184	20.24	357	305	33.55	Axilla; probably mammary
		41, F	353	301	33.11	353	301	33.11	Mammary area; small
		47, F	262	223	24.53	262	223	24.53	Mammary; cystic, large
		48, F	345	294	32.34	345	294	32.34	Mammary
04	1.7	01, M	223	216	367.2	223	216	367.2	Caecal area; several small nodules
		18, M	233	226	384.2	233	226	384.2	Kidney
		29, F	209	204	346.8	209	204	346.8	Caecal area; nodular

(a) *Findings in Rats That Died During the Experimental Period.* (1) *Spontaneous Diseases of an Inflammatory Nature.* Spontaneous inflammatory diseases encountered in this experiment were, for the most part, no different in their histological manifestations from those seen in rats in general. Most of these lesions have been quite well described by Ratcliffe,[2] and detailed descriptions will not be included here. In this section, emphasis will be placed on frequency of occurrence rather than on such descriptions.

Table 17.8 — Location and Percentage of Tumors in the Various Groups of Rats

	Location and number of tumors			Percentage with tumors		
Group	Internal	External	Total	Internal	External	Total
00	3	2	5	6	4	10
01	12	1	13	24	2	26
02	6	3	9	12	6	18
03	12	9	21	24	18	42
04	3	0	3	6	0	6

Table 17.9 — Rats Submitted for Microscopic Examination

			Number of rats sacrificed at indicated time after last treatment					
Group	Dose, n/day	Number of exposure-period deaths	Immediately after	1 month	2 months	3 months	Number of post-exposure deaths	Total
00	Control	16	12	6	4	6	4	48
01	0.12	19	11	7	4	7	2	50
02	0.06	17	12	7	4	8	2	50
03	0.11	18	10	8	4	6	2	48
04	1.7	48	0	0	0	0	0	48

The most common single type of inflammatory lesion observed was pulmonary infection, which occurred in 25 of the 48 rats in group 04 (52.1 per cent), 8 of 18 in group 03 (44.4 per cent), 5 of 17 in group 02 (29.4 per cent), 9 of 18 in group 01 (50 per cent), and 5 of 17 in the control group (29.4 per cent). The relation of the occurrence of this type of lesion to survival time and the degree of severity of the lesions in each case are given in Tables 17.10 to 17.15. In those cases designated as mild (□), there was usually a bronchitis or a bronchitis associated with mild bronchopneumonia. The moderate (■) and more severe cases (△ and ▲) showed correspondingly more severe pneumonias and/or bronchiectatic abscess formation.

Table 17.10 — Findings in Control Rats That Died During Treatment Period[a]

No. of treatments	47	66	69	98	135	152	166	173	189	241	245	267	283	308			Frequencies		
Animal No.	42	34	11	1	7	38	30	6	36	3	32	17	37	14	27	4			
Sex	F	F	M	M	M	F	F	M	F	M	F	M	F	M	F	M			
Pulmonary infection					△		△				△		■		□		5/16		
Necrosis, liver																	0/16		
Chronic nephritis																	0/16		
Nodular periarteritis																	0/16		
Ulcer																			
Small bowel					b			b	b								0/13		
Large bowel					b			b	b								0/13	0/16	
Edema, caecal nodes																	0/16		
Granuloma																			
Liver																	0/16		
Spleen														b			0/15		
Hemorrhage																			
Bone marrow			b														0/15		
Lymph node			b	b		b	b	b			b		b	b	b		0/7		
Adrenal	b	■			□		■		■	b	■						5/14		
Hyperplasia, splenic pulp		■	□		■	■	■		□	c		c	△	b	△	□	9/13		
Hypoplasia																			
Splenic follicles			□	□	□	□	□	□	□	c	■	c		b		□	9/13		
Lymph follicles	+	+	b	b	+	b	b	b		c	b	c	b	b	b		3/5		
Bone marrow			b									c					0/14		
Atrophy, testis												△		■			2/8		
Lack of ovarian follicles							b				b						0/6		
Reticulum-cell sarcoma										+		+					2/16	2/16	2/1
Lymphosarcoma																	0/16		
Lymphoma																	0/16		
Other sarcoma																	0/16		
Carcinoma																			
Lung																	0/16	0/16	
Uterus																	0/8		
Skin																	0/16		
Ovary																	0/8		
Leukemia																			
Lymphatic																	0/16	0/16	
Myelogenous																	0/16		
Fibroadenoma, mammary																	0/8	0/16	
Other benign tumors																	0/16		
Miscellaneous	d	e	f					d	d	e		d		f	b				

[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; and △, moderately severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Pulmonary hemorrhage.

[e] Pulmonary edema.

[f] Peritonitis.

Table 17.11 — Findings in Dying Rats Exposed to Neutron Radiation at 0.012 n per Treatment[a]

No. of treatments	51	60	69	79	92	100	125	160	170	250	265	268	273	273	289	303	305	309		
Animal No.	10	18	50	2	9	4	32	40	17	44	48	14	39	1	25	46	19	8	11	Frequencies
Sex	M	M	F	M	M	M	F	F	M	F	F	M	F	F	F	F	M	M	M	
Pulmonary infection		△	■	△	△					□	□	△	△	△	■	△	■			12/19
Necrosis, liver								b	■	b							■			12/17
Chronic nephritis										■	□	■	□	b						4/18
Nodular periarteritis																				0/19
Ulcer																				
Small bowel																				0/19
Large bowel																				0/19 }
Edema, caecal nodes																				0/19 } 2/17
Granuloma																				
Liver			□					b		b										1/17 }
Spleen					b			△					b							1/17 }
Hemorrhage																				
Bone marrow																			b	0/18
Lymph node	b			b							b	b		b	b	b		b		0/11
Adrenal	b							b	b	b	■	b			■	■				3/14
Hyperplasia, splenic pulp	□	□	□	△	b	▲				△	△	■	b		■	□	△	□	■	13/17
Hypoplasia																				
Splenic follicles		□	□		b				■	□			b	■		□		■	□	8/17
Lymph follicles	b	+	+	b			c	+	c	c	b	b	+	b	b	b		b	c	4/7
Bone marrow									c										b	0/17
Atrophy, testis					■															1/10
Lack of ovarian follicles								b												0/8
Reticulum-cell sarcoma							+		+	+									+	4/19 }
Lymphosarcoma																				0/19 } 4/19
Lymphoma																				0/19 }
Other sarcoma																				0/19
Carcinoma																				
Lung																				0/19 } 4/19
Uterus																				0/9 }
Skin																				0/9 } 0/19
Ovary																				0/9 }
Leukemia																				
Lymphatic																				0/19 } 0/19
Myelogenous																				0/19 }
Fibroadenoma, mammary																				0/9 } 1/19
Other benign tumors																				1/19 }
Miscellaneous						+, d														

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[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Subcutaneous fibroma.

Table 17.12 — Findings in Dying Rats Exposed to Neutron Radiation at 0.06 n per Treatment[a]

No. of treatments	10	64	70	115	122	158	160	178	211	227	239	251	262	276	280	296	310			
Animal No.	2	19	24	14	18	26	25	47	49	13	35	22	6	1	8	16	17	Frequencies		
Sex	M	F	M	M	M	F	F	F	F	M	F	M	M	F	M	M	M			
Pulmonary infection			▲					△							■	■	■	5/17		
Necrosis, liver		■				■												2/17		
Chronic nephritis														△				1/17		
Nodular periarteritis																		0/17		
Ulcer																				
Small bowel										△								1/17		
Large bowel													b			c	△	1/15	1/17	
Edema, caecal nodes																		0/17		
Granuloma																				
Liver																		0/17		
Spleen							c											0/16		
Hemorrhage																				
Bone marrow																		0/17		
Lymph node		△	c	c	c	c	c	c		c					c	c	c	1/7		
Adrenal											c						c	0/15		
Hyperplasia, splenic pulp			□	□	□	□	c	□	▲	△		d	△	d		□		10/14		
Hypoplasia																				
Splenic follicles		△				□	c	□	□		□	d		d		□		6/14		
Lymph follicles			c	c	c	c	c	c	d	c	+	d	d	d	c	c	c	1/3		
Bone marrow									d			■	d					1/15		
Atrophy, testis	c		e							△								1/9		
Lack of ovarian follicles								c										0/6		
Reticulum-cell sarcoma									+			+	+	+				4/17	4/17	4/17
Lymphosarcoma																		0/17		
Lymphoma																		0/17		
Other sarcoma																		0/17		
Carcinoma																				
Lung																		0/17	0/17	
Uterus																		0/7		
Skin																		0/17		
Ovary																		0/7		
Leukemia																				
Lymphatic																		0/17	0/17	
Myelogenous																		0/17		
Fibroadenoma, mammary																		0/7	0/17	
Other benign tumors																		0/17		
Miscellaneous	f	g	h	i	j	f	g	k	f,l	f,k	f									

[a] The following symbols denote the varying degrees of injury; +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] Bowel ulceration associated with reticulum-cell sarcoma.

[c] No section examined.

[d] Changes not recorded because of involvement by tumor.

[e] Mild testicular atrophy, severe acute inflammation of scrotum.

[f] Pulmonary edema.

[g] Liver parasites.

[h] Peritonitis.

[i] Inflammation of large-bowel submucosa.

[j] Retroperitoneal hemorrhage and hemorrhage in renal pelvis; purulent exudate in subarachnoid space, lumbar spine; clinical paralysis of hindquarters.

[k] Pericarditis.

[l] Hemorrhage and ulceration in stomach.

Table 17.13 — Findings in Dying Rats Exposed to Neutron Radiation at 0.11 n per Treatment[a]

No. of treatments	158	160	197	202	215	217	222	222	226	242	249	260	283	292	292	299	300	303	Frequencies		
Animal No.	49	17	10	34	15	45	47	12	26	38	8	6	4	28	48	41	33	35			
Sex	F	M	M	F	M	F	F	M	F	F	M	M	M	F	F	F	F	F			
Pulmonary infection					△	■	■			△			△	□	■	△			9/18		
Necrosis, liver																			0/18		
Chronic nephritis																		■	1/18		
Nodular periarteritis																			0/18		
Ulcer																					
Small bowel																b			0/17		
Large bowel														△		b			1/17		
Edema, caecal nodes																			0/18	2/18	
Granuloma																					
Liver														△					1/18		
Spleen																			0/18		
Hemorrhage																					
Bone marrow																			0/18		
Lymph node										b			b			b	b		0/14		
Adrenal																▲			1/18		
Hyperplasia, splenic pulp			△	□	△	□	△	△		■	c	□	□	d		□	□	□	12/16		
Hypoplasia																					
Splenic follicles	□	■		△		□			■	□	c	□	□	d		□	□	□	11/16		
Lymph follicles	c	c	c		d	c		c	c	b	c		b	d	+	b	b		1/5		
Bone marrow	c		c		d				c		c			d					0/12		
Atrophy, testis		b	□								△								2/6		
Lack of ovarian follicles	b			+		+										b		+	3/9		
Reticulum-cell sarcoma	+	+	+	+		+			+		+								7/18		
Lymphosarcoma								+											1/18	8/18	
Lymphoma																			0/18		
Other sarcoma												+,e							1/18		
Carcinoma																					
Lung																			0/18		13/18
Uterus																			0/11	2/18	
Skin							+,f											+,f	2/18		
Ovary																			0/11		
Leukemia																					
Lymphatic																			0/18	2/18	
Myelogenous					+									+					2/18		
Fibroadenoma, mammary															+	+			2/11	2/18	
Other benign tumors																			0/18		
Miscellaneous											g										

[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of involvement by tumor.

[d] Changes not recorded because of leukemic involvement.

[e] Osteogenic sarcoma.

[f] Mammary carcinoma.

[g] Inflammation of large-bowel submucosa.

Table 17.14 — Findings in Dying Rats Exposed to Neutron Radiation at 1.7 n per Treatment[a]

No. of treatments	24	26	76	80	112	122	166	172	173	174	177	179	180	181	184	186	186	190
Animal No.	2	46	21	3	24	42	9	10	27	25	18	11	48	26	16	19	33	17
Sex	M	F	M	M	M	F	M	M	F	M	M	M	F	F	M	M	F	M
Pulmonary infection	△	■	■	△	△	△		△	■	△		■		△				△
Necrosis, liver						■												
Chronic nephritis																		
Nodular periarteritis																		
Ulcer																		
Small bowel									b				b					b
Large bowel									b		b		b					b
Edema, caecal nodes	■																	
Granuloma																		
Liver	■				□						△							
Spleen	▲						b				■					b		
Hemorrhage																		
Bone marrow					□													
Lymph node						b	b	b	b	b	b	b						b
Adrenal						□			b		b		■	■	□			
Hyperplasia, splenic pulp		■	□		■	■	b	□	c		c	□	□			b	c	
Hypoplasia																		
Splenic follicles	□	■	■	■	■	■	b	△	c	■	c	△	■	■	△	b	c	■
Lymph follicles		+	+	+	+	b	b	b	b	b	b	b		b	b			b
Bone marrow					□		□		c		c						c	
Atrophy, testis	■		▲	▲	▲		▲	▲		▲	▲	▲			▲	▲		▲
Lack of ovarian follicles									b				e	+			+	
Reticulum-cell sarcoma													+					
Lymphosarcoma																		
Lymphoma																		
Other sarcoma																		
Carcinoma																		
Lung																		
Uterus																		
Skin or mammary																		
Ovary																		
Leukemia																		
Lymphatic																		
Myelogenous									+		+						+	
Fibroadenoma, mammary																		
Other benign tumors																		
Miscellaneous					g	g												

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[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement by reticulum-cell sarcoma.

[e] Decrease in ovarian follicles.

[f] Bronchial adenoma.

[g] Liver, encysted parasites (cestode).

[h] Moderate pulmonary hemorrhage.

Table 17.14 — (Continued)

No. of treatments	194	194	200	200	201	202	203	203	205	206	212	214	214	214	215	218	219
Animal No.	5	37	30	31	23	29	13	45	32	20	34	50	22	12	1	7	4
Sex	M	F	F	F	M	F	M	F	F	M	F	F	M	M	M	M	M
Pulmonary infection		■		□	□		□	△	■	□	□	■	■			△	
Necrosis, liver						■											
Chronic nephritis																	
Nodular periarteritis																	
Ulcer																	
Small bowel																	
Large bowel					△								△				△
Edema, caecal nodes																	
Granuloma																	
Liver					■								□				□
Spleen																	
Hemorrhage																	
Bone marrow										□							
Lymph node	b		b	b	b		b	b	b	b	b	△			b	b	b
Adrenal		b				□		□	△		■						b
Hyperplasia, splenic pulp		□	c	□	■	▲	□	□	□	□	□	△	▲	▲	c	c	□
Hypoplasia																	
Splenic follicles	△	■	c	□	△	△	△	△	△	△	■	△	■	■	c	c	△
Lymph follicles	b		b	b	b	d	b	b	b	b	b	+		+	b	b	b
Bone marrow			c							■					c	c	
Atrophy, testis	▲				▲		▲			▲				▲	▲	▲	▲
Lack of ovarian follicles		b	+	b				e	+		e	b					
Reticulum-cell sarcoma																	
Lymphosarcoma																	
Lymphoma																	
Other sarcoma																	
Carcinoma																	
Lung																	
Uterus																	
Skin or mammary																	
Ovary																	
Leukemia																	
Lymphatic																	
Myelogenous			+														
Fibroadenoma, mammary														+	+	+	
Other benign tumors																	
Miscellaneous																	

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[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement by reticulum-cell sarcoma.

[e] Decrease in ovarian follicles.

[f] Bronchial adenoma.

[g] Liver, encysted parasites (cestode).

[h] Moderate pulmonary hemorrhage.

Table 17.14— (Continued)

No. of treatments	220	222	222	223	224	224	226	229	229	239	249	251	281			
Animal No.	39	35	15	14	8	41	49	38	44	47	40	6	43	Frequencies		
Sex	F	F	M	M	M	F	F	F	F	F	F	M	F			
Pulmonary infection		△										□		25/48		
Necrosis, liver														2/47		
Chronic nephritis														0/48		
Nodular periarteritis														0/48		
Ulcer																
Small bowel														0/45	8/48	
Large bowel														3/44		
Edema, caecal nodes														1/48		
Granuloma																
Liver											□			7/48		
Spleen			▲											3/46		
Hemorrhage																
Bone marrow														2/48		
Lymph node	b	b	b	b	b		b	b		b	b	b		1/16		
Adrenal		■				▲	△	△	■	■	■		△	16/44		
Hyperplasia, splenic pulp	■	□	■	△	c	■		■	■		□		□	28/39		
Hypoplasia																
Splenic follicles	△	△	■	■	c	■	△	△	△	▲	△	■	▲	39/39		
Lymph follicles	b	b	b	b	b		b	b	+	b	b	b		7/16		
Bone marrow					c									3/41		
Atrophy, testis			▲	▲	▲							▲		24/24		
Lack of ovarian follicles	b	b				+	+	+	b	+	b		+	9/16		
Reticulum-cell sarcoma														2/48	2/48	11/48
Lymphosarcoma														0/48		
Lymphoma														0/48		
Other sarcoma														0/48		
Carcinoma																
Lung														1/48	1/48	
Uterus														0/23		
Skin or mammary														0/48		
Ovary														0/23		
Leukemia																
Lymphatic														0/48	8/48	
Myelogenous														8/48		
Fibroadenoma, mammary			+											0/23	0/48	
Other benign tumors														0/48		
Miscellaneous			h						f							

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[a] The following symbols denote the varying degrees of injury: +, trace; □, mild; ■, moderate; △, moderately severe; and ▲, severe.

[b] No section examined.

[c] Changes not recorded because of leukemic involvement.

[d] Changes not recorded because of involvement by reticulum-cell sarcoma.

[e] Decrease in ovarian follicles.

[f] Bronchial adenoma.

[g] Liver, encysted parasites (cestode).

[h] Moderate pulmonary hemorrhage.

Table 17.15 — Findings in Neutron-radiated Rats Sacrificed After 311 Treatments[a]

Dose per treatment	Controls													0.0117 n											
Animal No.	40	16	2	19	18	31	39	45	48	9	25	35	Freq.	16	35	28	7	15	21	31	34	42	45	3	Freq.
Sex	F	F	M	M	M	F	F	F	F	M	F	F		M	F	F	M	M	M	M	F	F	F	M	
Pulmonary infection			☐						■			☐	3/12										△		1/11
Necrosis, liver																									
Chronic nephritis																									
Nodular periarteritis																									
Ulcer																									
Small bowel																									
Large bowel																△					△				2/11
Edema, caecal nodes																△									1/11
Granuloma																									
Liver																■									1/11
Spleen																									
Hemorrhage																									
Bone marrow																									
Lymph node		b	b		b	b	b	b	b	b	b	b	0/2	b	b		b	b	b	b	b	b	b	b	0/1
Adrenal																☐									1/11
Hyperplasia, splenic pulp									■	☐	■	☐	4/12	☐						☐					2/11
Hypoplasia																									
Splenic follicles																									
Lymph follicles	c	b	b	c	b	b	b	b	b	b	b	b	0/0	b	b		b	b	b	b	b	b	b	b	0/1
Bone marrow																									
Atrophy, testis																									
Lack of ovarian follicles		b									b	b	0/5			b									0/4
Reticulum-cell sarcoma	+			+									2/12												
Lymphosarcoma																									
Lymphoma																									
Other sarcoma																									
Carcinoma																									
Lung																									
Uterus																									
Skin																									
Ovary																									
Leukemia																									
Lymphatic																									
Myelogenous																									
Fibroadenoma, mammary		+											1/8												
Other benign tumors									e																
Miscellaneous																						f			

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[a] The following symbols are used to denote degree of injury: +, trace; ☐, mild; ■, moderate; and △, moderately severe.

[b] No sections examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Neurofibroma, ear.

[f] Cystic disease, mammary gland.

[g] Moderate fatty degeneration, liver.

Table 17.15 — (Continued)

Dose per treatment	0.058 n													0.107 n										
Animal No.	41	44	46	34	36	40	10	23	7	28	9	12	Freq.	36	44	27	46	16	18	3	5	7	22	Freq.
Sex	F	F	F	F	F	F	M	M	M	F	M	M		F	F	F	F	M	M	M	M	M	M	
Pulmonary infection	□				■		■						3/12						■				■	2/10
Necrosis, liver																								
Chronic nephritis			□	□	□								3/12											
Nodular periarteritis																								
Ulcer																								
Small bowel																								
Large bowel																								
Edema, caecal nodes																								
Granuloma																								
Liver																								
Spleen			b										0/11											
Hemorrhage																								
Bone marrow																								
Lymph node	b	b	b	b	b	b	b	b	b	b	b	b	0/0	b	b									0/8
Adrenal	■		■	b	△			b		b			3/9	b			■	b						1/8
Hyperplasia, splenic pulp	□	□	b		□				□	□	□	□	7/11			■		□	■	□				4/10
Hypoplasia																								
Splenic follicles			b										0/11											4/10
Lymph follicles	b	b	b	b	b	b	b	b	b	b	b	b	0/0	b	b	d	c		+	b	b	b	b	1/2
Bone marrow																								
Atrophy, testis																							□	1/6
Lack of ovarian follicles										b			0/6											
Reticulum-cell sarcoma																+	+							2/10
Lymphosarcoma																								
Lymphoma																								
Other sarcoma																								
Carcinoma																								
Lung																								
Uterus																								
Skin																								
Ovary																								
Leukemia																								
Lymphatic																								
Myelogenous																								
Fibroadenoma, mammary				+									1/7											
Other benign tumors																								
Miscellaneous														g										

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[a] The following symbols are used to denote degree of injury: +, trace; □, mild; ■, moderate; and △, moderately severe.

[b] No sections examined.

[c] Changes not recorded because of involvement by reticulum-cell sarcoma.

[d] Changes not recorded because of involvement by lymphosarcoma.

[e] Neurofibroma, ear.

[f] Cystic disease, mammary gland.

[g] Moderate fatty degeneration, liver.

Paratyphoid infection or a paratyphoidlike infection occurred in a few of the rats in one or another of its various forms. Chronic ulcerative caecitis occurred in three rats in group 04 (6.2 per cent) and in one case each in groups 02 (2.0 per cent) and 03 (2.1 per cent). Those in group 04 were all accompanied by the characteristic small necrotic granulomatous lesions in the liver. These hepatic lesions were observed in four additional cases in group 04, making a total of seven cases, or 14.6 per cent of the rats that died in this group. Similar lesions which were unusually severe were seen in the spleen in two cases. Group 01 showed these focal necrotic lesions in only two cases (one in the spleen and one in the liver). The one case in group 03 with ulcerated bowel had spleen and liver necroses. In spite of the lack of bacteriological studies in these animals, these lesions are assumed to be related to a paratyphoid infection on the basis of bacteriological studies on other animals from the colony in this laboratory[3] and studies reported by Buchbinder and others[4] on animals showing identical lesions. Although the exact etiological agent is not definitely known in the cases in this experiment, it is well known that identical lesions occur quite frequently in the nonradiated rats.[5]

Chronic nephritis occurred sporadically in these rats. In group 01, four cases, or 8.0 per cent, showed a mild or moderate chronic inflammation as did one case in both groups 02 and 03.

In addition to the above-mentioned lesions, there were found occasionally in the various groups cases with mild to moderate focal necrosis in the liver, cases with peritonitis or pericarditis, cases with pulmonary edema, and cases with encysted cestodes in the liver (see Tables 17.10 to 17.14).

(2) Changes in Cellularity of Hematopoietic Organs and Gonads. Spleen. In general, two different types of alteration were observed in the spleens of these rats:

1. Hypoplasia of the splenic follicles, characterized by a general reduction in size and cellularity of the Malpighian bodies throughout the section of spleen. Few spleens in any groups showed the large active follicles commonly observed in young healthy rats (Fig. 17.3). Hypoplasia was a common occurrence in the controls and in the three lower-dosage groups, occurring in 9 of 13 controls (69.2 per cent), 8 of 17 in group 01 (47.1 per cent), 6 of 14 in group 02 (42.9 per cent), and 11 of 16 in group 03 (68.6 per cent), but, for the most part, in these groups the change was mild in degree, and the few rats with more marked hypoplasia almost invariably showed severe congestion of the splenic pulp or massive invasion of the splenic pedicle by tumor. Group 04, on the other hand, showed hypoplasia of the splenic follicles in 100 per cent of the 39 cases, and all but 2 of these

cases were moderate to severe in degree (see Fig. 17.4 and Table 17.14); only 3 of these cases were complicated by congestion. There was no clear-cut progression of this change with respect to the time of death, although an arbitrary division of group 04 at the 200-treatment point shows a distribution of the different grades of damage which seems significant (see Table 17.16).

2. A diffuse cellular hyperplasia of the splenic pulp was a common occurrence in rats in all groups, including the controls. This change showed no apparent relation to time, dosage, or other lesions, and the significance of the change is not known. For the most part, the cell types contributing to this hyperplasia were large and small lymphocytes, mature granulocytic leucocytes, and immature granulocytes and erythrocytes.

Lymph Nodes. Hypoplasia of the cortex of the regional lymph nodes was observed in many of the cases from which sections were obtained, and which were free from leukemic and tumor involvement. The small number of such cases, however, precludes any interpretation of the findings.

Bone Marrow. There were no changes observed in the bone marrow which were considered to be significant. No importance can be attached to the very small number of cases in which the marrow was slightly less cellular than that usually seen.

Testes. Testicular atrophy or, more exactly, a decrease in the number of germinal elements in the seminiferous tubules occurred in an occasional male animal in the control group and in the three lower-dosage groups of experimental animals. In all these cases the reduction in the number of germinal cells was incomplete and affected the more mature germinal elements, i.e., spermatozoa, spermatids, and, in the more severe cases, spermatocytes. Degenerative changes in spermatids and abnormal multinucleated forms were occasionally seen (Fig. 17.5). In the vast majority of the rats in these groups the testes were normal in every way. In striking contrast to these findings, there was testicular damage in all male rats in group 04, and, with the exception of one animal (No. 2) which died after only 24 treatments, the reduction in the number of germinal cells amounted to complete or practically complete depletion (Fig. 17.6). In the one case which showed partial atrophy the reduction in germinal elements was, for the most part, in the earlier stages, i.e., spermatogonia were almost completely absent, and there was a marked reduction in the number of primary spermatocytes (Fig. 17.7).

Secondary to the depletion of germinal elements in the testes of male rats in group 04, there were a marked reduction in size of the seminiferous tubules and a depletion of spermatozoa from the epididymides. Animal 2 showed no change in the contents of the epididy-

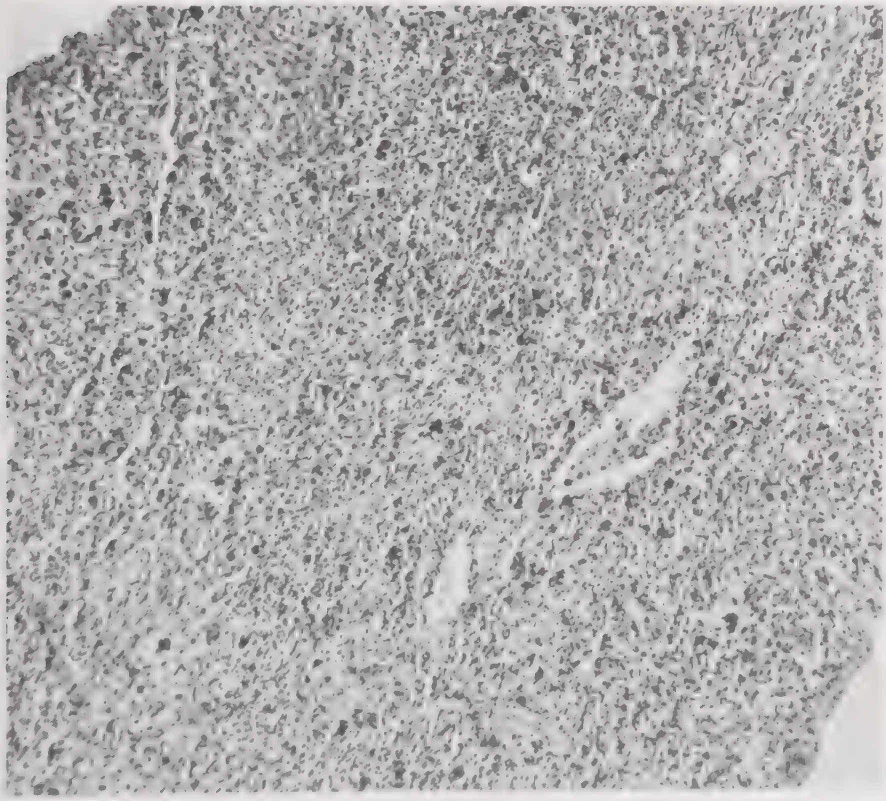

Fig. 17.3—Hypoplasia of the rat spleen.

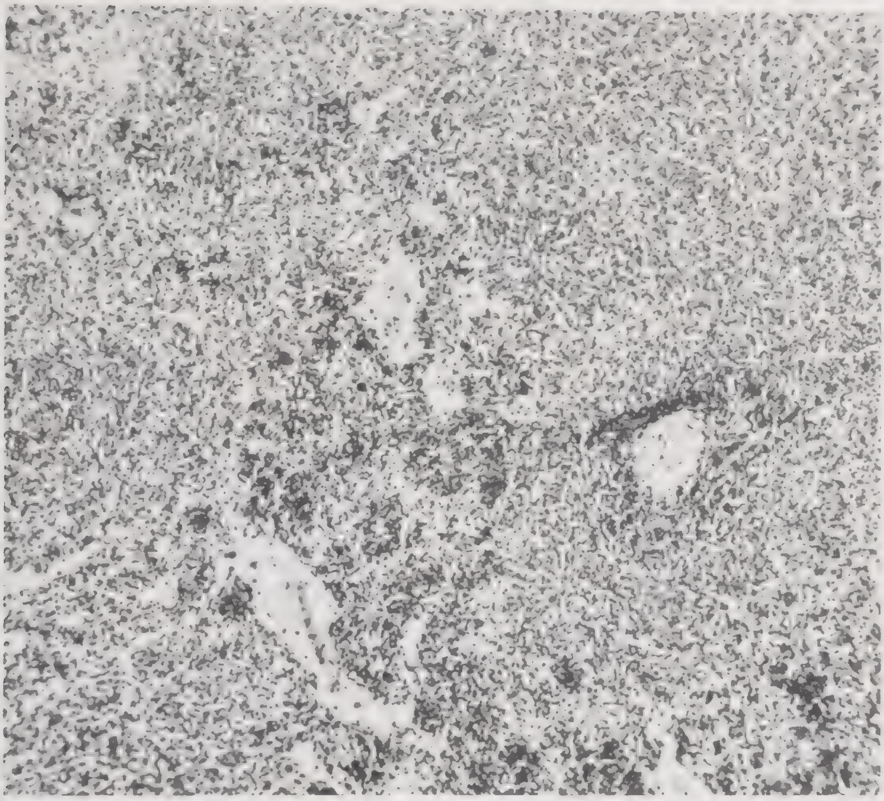

Fig. 17.4—Hypoplasia of the rat spleen.

mis, whereas animals 46, 21, and 3, all of which died before the hundredth treatment, showed spermatozoa only in the most distal parts of the epididymides. Rats that died after the hundredth treatment showed no spermatozoa in the two sections of epididymides which were examined in each case. Sertoli nuclei were present in the seminiferous tubules in all cases, although the rats that died after the hundredth treatment showed varying numbers of Sertoli nuclei which were distorted and pycnotic.

Table 17.16—Distribution of Various Grades of Splenic Hypoplasia in Rats Exposed to 1.7 n/Day

Number of treatments	Total cases	Mild	Moderate	Moderately severe	Severe
200 or less	16	2	10	4	0
>200	32	0	7	14	2

Ovaries. The histological picture of the normal rat ovary is quite varied, depending on the stage of the cycle and the particular part of the ovary examined, and no significance can be attached to the appearance of a single section of ovary from an isolated animal. It was not practical in this experiment to examine serial sections from the ovaries of all animals. The procedure employed was to make two or three sections from one ovary from each case. The ovaries so examined from rats (without exception in groups 01 and 02 and in the control group, and with one exception in group 03) showed at least one, and usually several, normal growing follicles in addition to primordial follicles and atretic follicles. Corpora lutea were present in many cases. In contrast to this picture the female rats of group 04, with few exceptions, showed no normal growing follicles in the two sections examined (Fig. 17.8). Although growing follicles were not observed, some of the sections showed small masses of cells of the type seen in corpora lutea, and primordial follicles were present. Atretic follicles were quite numerous in most of these cases, and occasionally a small follicle was observed in which the granulosa cells were undergoing degeneration. Usually the sections were quite small, and it will be noted that most of the ovaries in the group 04 female rats and a few of those in group 03 were much smaller than normal. In order to rule out the possibility that chance sectioning in these cases had been responsible for these differences, the remainder of the block in each case was sectioned serially and every tenth section was examined. This supplementary study revealed that the observations made on the two sections can be considered to be repre-

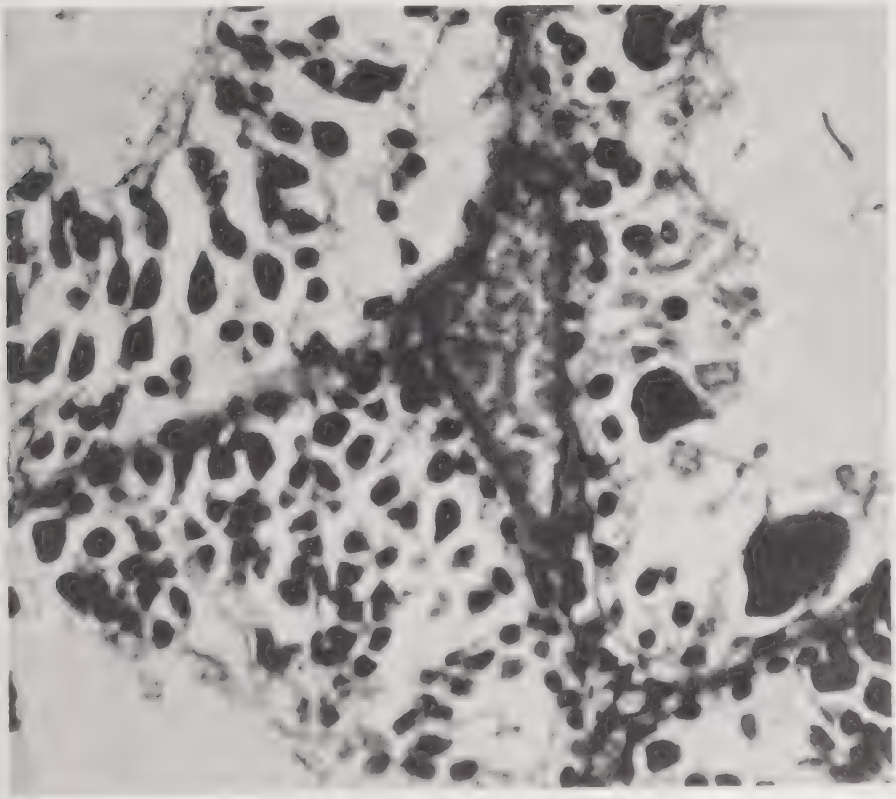

Fig. 17.5—Degenerative changes in the testes of rats.

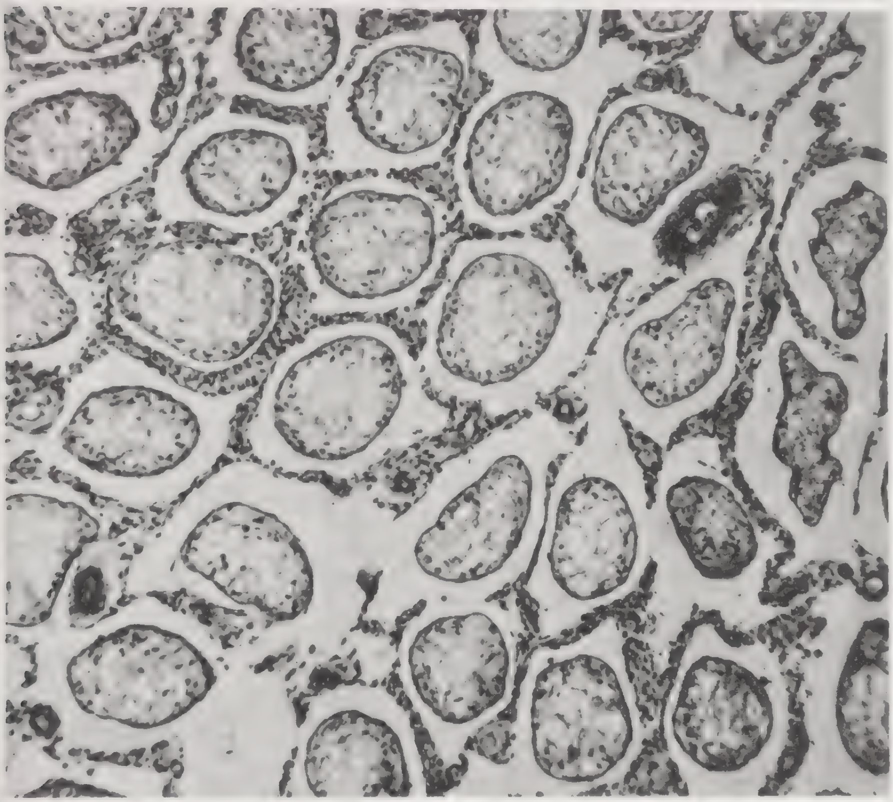

Fig. 17.6—Degenerative changes in the testes of rats.

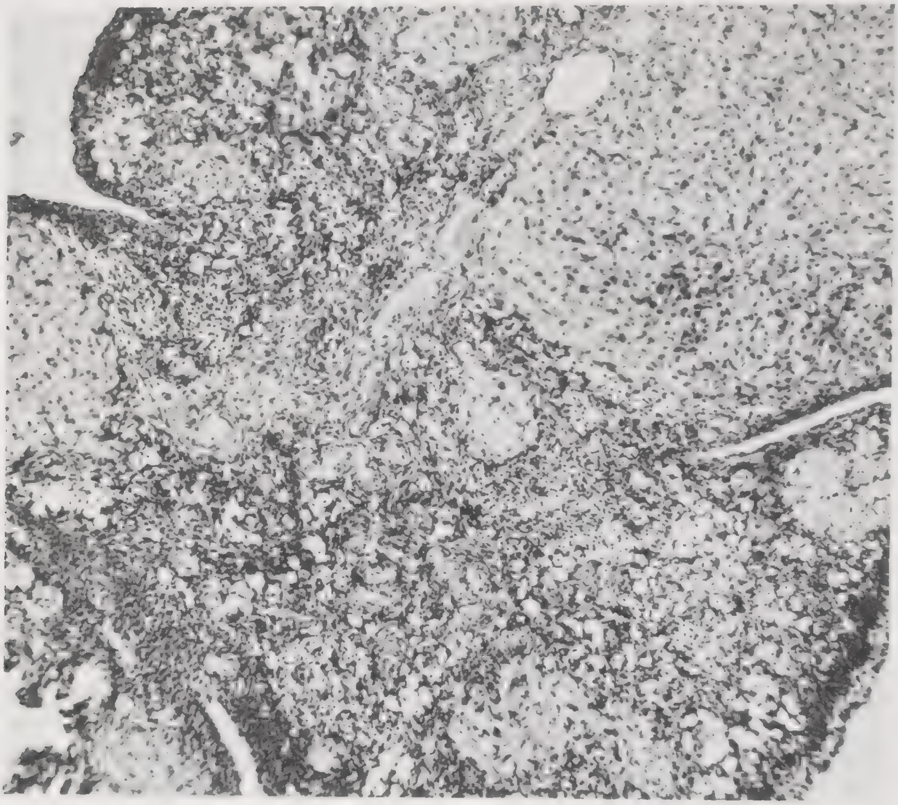

Fig. 17.8—Degenerative changes in the ovaries of rats.

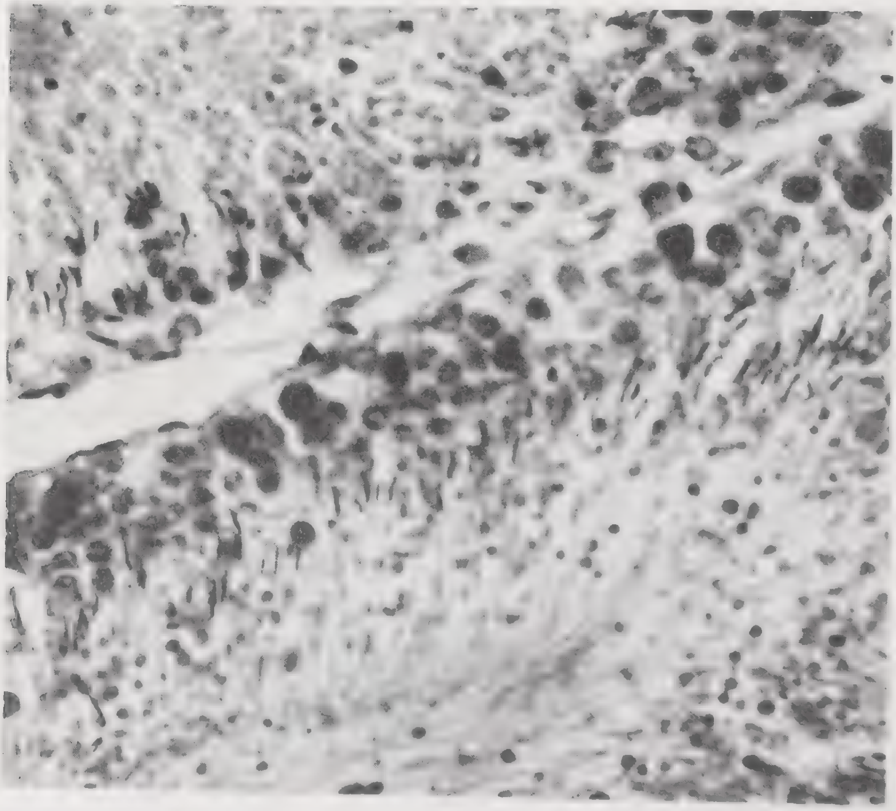

Fig. 17.7—Degenerative changes in the testes of rats.

sentative of those in the organ as a whole. No growing or Graafian follicles were seen in any of these ovaries.

(3) Hemorrhagic Manifestations. Hemorrhagic changes occurred with significant frequency only in the adrenal. These changes were characterized by the development of cystic spaces filled with red blood cells or disintegrated red cells, usually with some accumulation of blood pigments in macrophages in the surrounding tissues. This occurred in 5 of 14 or 35.7 per cent of the controls, in 3 of 14 or 21.4 per cent of group 01, in none of group 02, in 1 of 18 or 5.6 per cent of group 03, and in 16 of 44 or 36.4 per cent of group 04. Although this change was apparently unrelated to dosage, the sex incidence is of interest. Of the 25 cases observed, only 2 cases were in males, and in both of these the condition was mild in degree.

Mild to moderate pulmonary hemorrhage occurred in four rats in the control group and in one rat of group 04. One case of lymph-node hemorrhage was observed in group 04.

(4) Neoplastic Disease. Benign Tumors. The occurrence of benign tumors was limited to two cases of fibroadenoma of the breast in group 03 rats, one case of subcutaneous fibroma in group 01, and one case with a bronchial adenoma in group 04.

Malignant Tumors. By far the most common type of malignant tumor observed in these rats was a reticulum-cell sarcoma. A total of 19 cases was encountered in the 119 rats from all groups that died during the exposure period. In all cases the mesenteric nodes were involved, and, in approximately half the cases, tumor masses were found in the mediastinum. In 13 cases the bone marrow of the vertebrae was infiltrated with tumor cells, and in 11 the femoral epiphysis and diaphysis were involved. Liver infiltration was noted in 9 cases. Pancreas, intestinal wall, ovary, and splenic pedicle were less frequently invaded. In a few cases there was slight involvement of the spleen. The microscopic appearance of this tumor was very characteristic, being composed of large round or polygonal cells with large round or slightly indented vesicular nuclei. Hemorrhage and necrosis in these tumor masses were frequently observed. Ascites was a common finding in these rats, occurring in 11 cases, and pleural effusion occurred in 6 of the 8 cases with mediastinal involvement. Figure 17.9 shows a section through one of these tumor masses. This type of tumor occurred in 2 of the 16 controls (12.5 per cent), in 4 of the 19 rats in group 01 (21.1 per cent), in 4 of the 17 in group 02 (23.5 per cent), in 7 of 18 in group 03 (38.9 per cent), and in 2 of 48 in group 04 (4.2 per cent).

Other malignant tumors were of various types. Two cases of duct carcinoma of the breast were observed in group 03 (Fig. 17.10). In one of these cases there were metastatic lesions in the lung. Also in

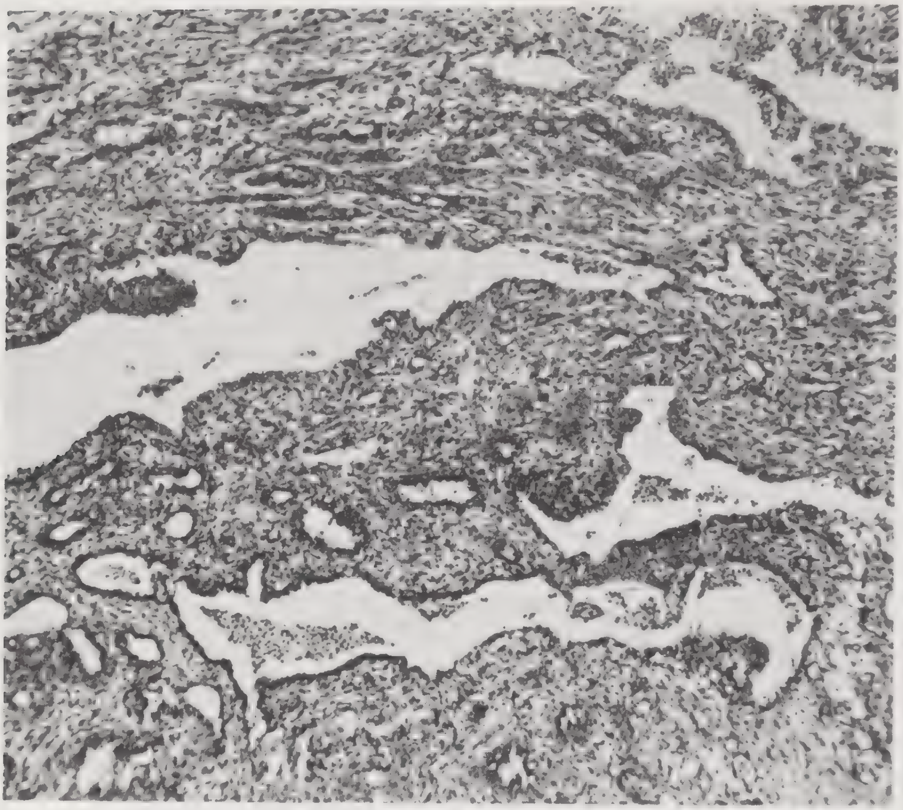

Fig. 17.10—Carcinoma of the breast in rats.

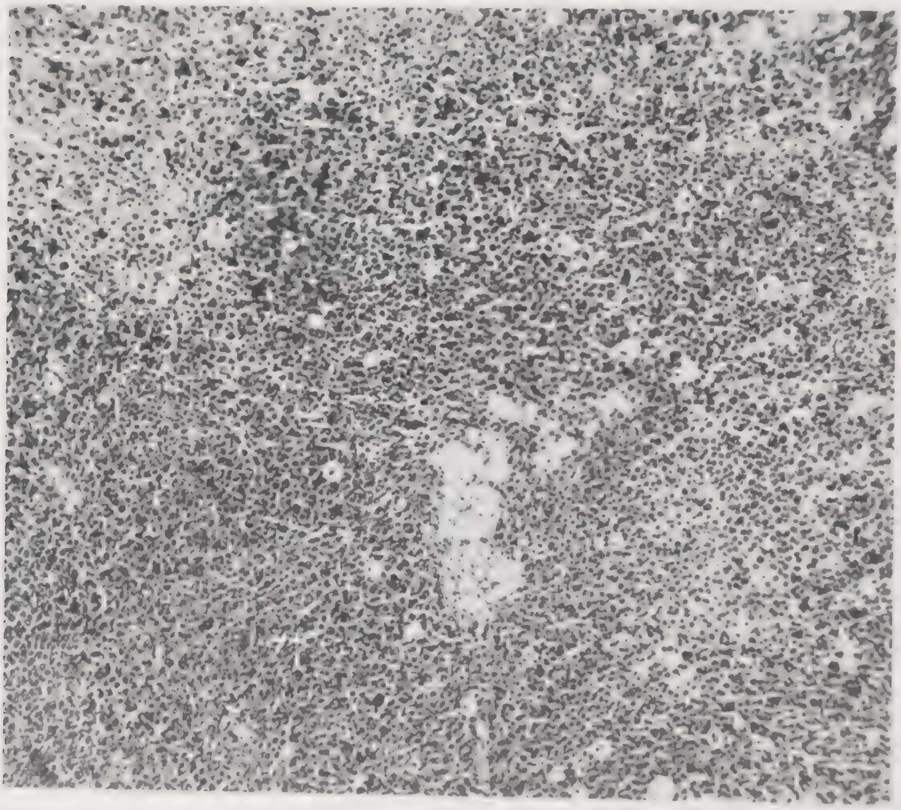

Fig. 17.9—Tumor (reticulum-cell sarcoma) in rats.

group 03 there was one case of osteogenic sarcoma (Fig. 17.11) and one case with a small-cell type of lymphosarcoma involving the inguinal lymph nodes and with metastatic nodules in the lung (Fig. 17.12).

Leukemia. In 10 rats in which histological examinations were made, there were observed changes which were strongly suggestive of leukemia. Because of the extreme difficulty in making a definite diagnosis of such conditions from routinely prepared sections in rats, it was decided to refer to the condition observed as a "leukemoid reaction."*

Figures 17.13 and 17.14 show high-magnification photomicrographs of typical cellular accumulations. Many of the cells, particularly in the more dense accumulations such as that in Fig. 17.14, are difficult to classify. These were large cells with large round or indented nuclei which were often slightly eccentric; one or more prominent nucleoli were often apparent, although some cells showed a diffuse chromatin arrangement. In addition, there were usually cells with all the characteristics of myelocytes and cells which were mature or almost mature polymorphonuclear leucocytes. The latter forms (Fig. 17.13) were quite numerous in loosely arranged accumulations such as those in the mesenteries and perirenal connective tissue. Besides the bone marrow, which was involved in all cases, the organs most commonly involved were spleen, liver, kidney, and mesenteries (Figs. 17.15 to 17.18). The accumulation of leucocytes in sections of heart and blood vessels was marked in some cases (Fig. 17.19). The various abnormalities which have a bearing on the diagnosis of leukemia are summarized in Table 17.17. Besides a marked similarity in cell type in the different animals, there was also a fairly consistent pattern of distribution of the various changes. Two cases were somewhat less clear-cut than the others. Number 18 in group 04 showed extensive and severe paratyphoid infection in the liver and spleen which tended to mask the possible leukemoid involvement. The presence of definite peripheral-blood changes in this case strengthen the diagnosis of leukemia. Rat 15 of group 03 showed evidence suggestive of leukemia in the marrow (Fig. 17.15), along with small cellular accumulations in the liver. Even in the absence of changes in the peripheral blood at the last blood count (approximately 1 month prior to death), the evidence available is considered sufficient to include this animal in the leukemoid group.

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* Editor's Note: Similar difficulty in establishing the differential diagnosis between leukemia and severe leucocytoses was encountered in the chronic X-ray experiments with rats. In that case a high incidence of severe infections in all groups appeared to have been largely responsible for the "leukemoid" blood picture (see Chap. 11).

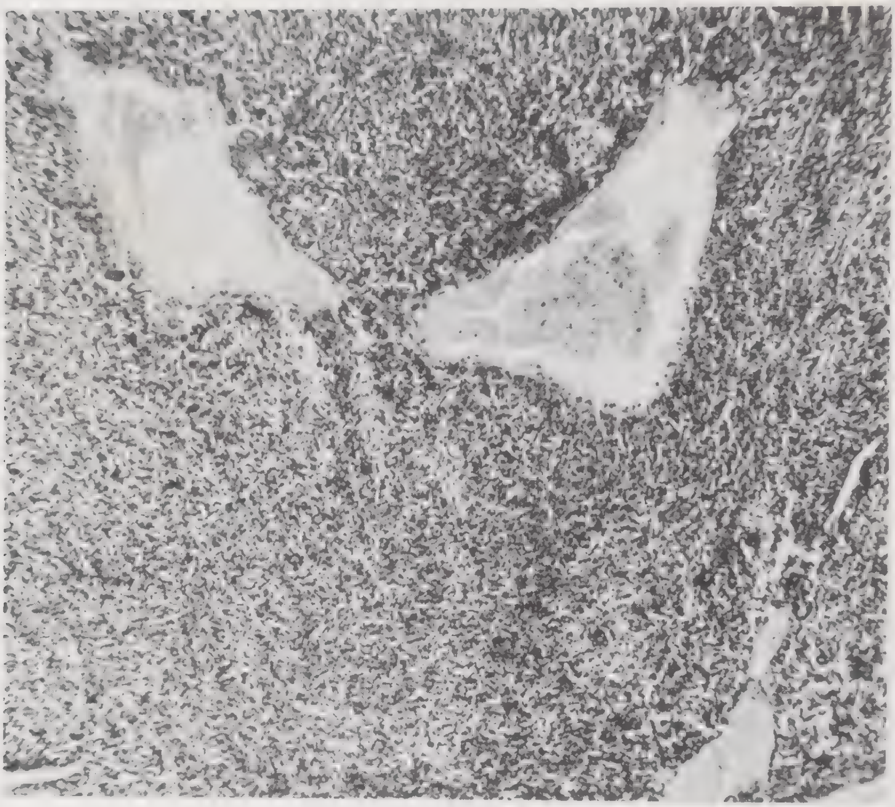

Fig. 17.12—Lymphosarcoma with metastases in the rat.

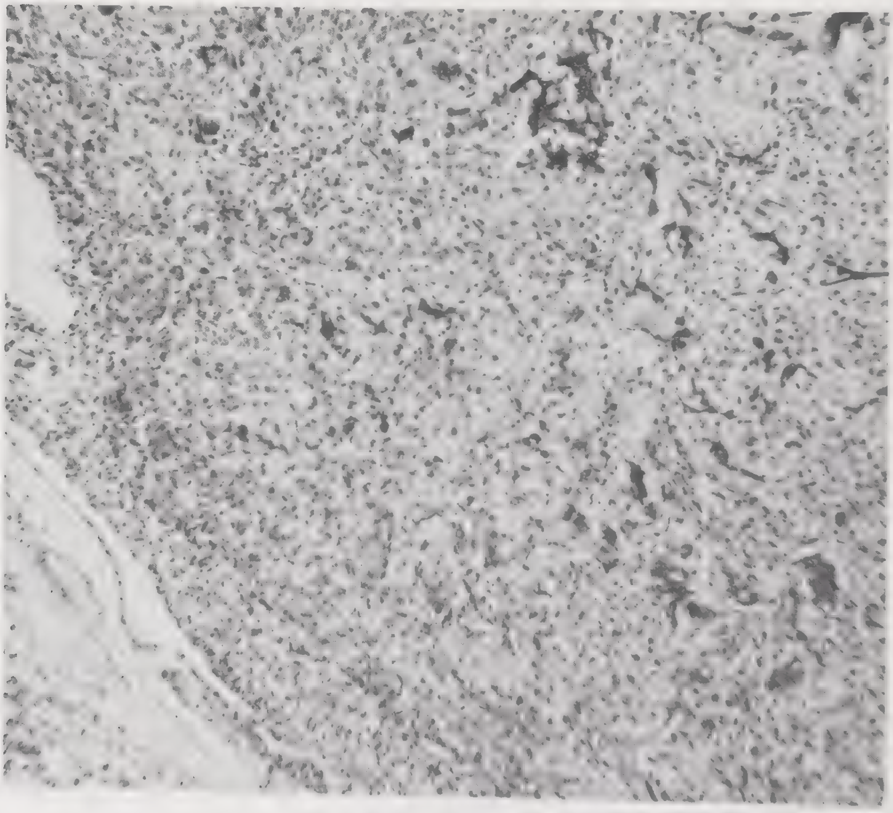

Fig. 17.11—Osteogenic sarcoma with metastases in rats.

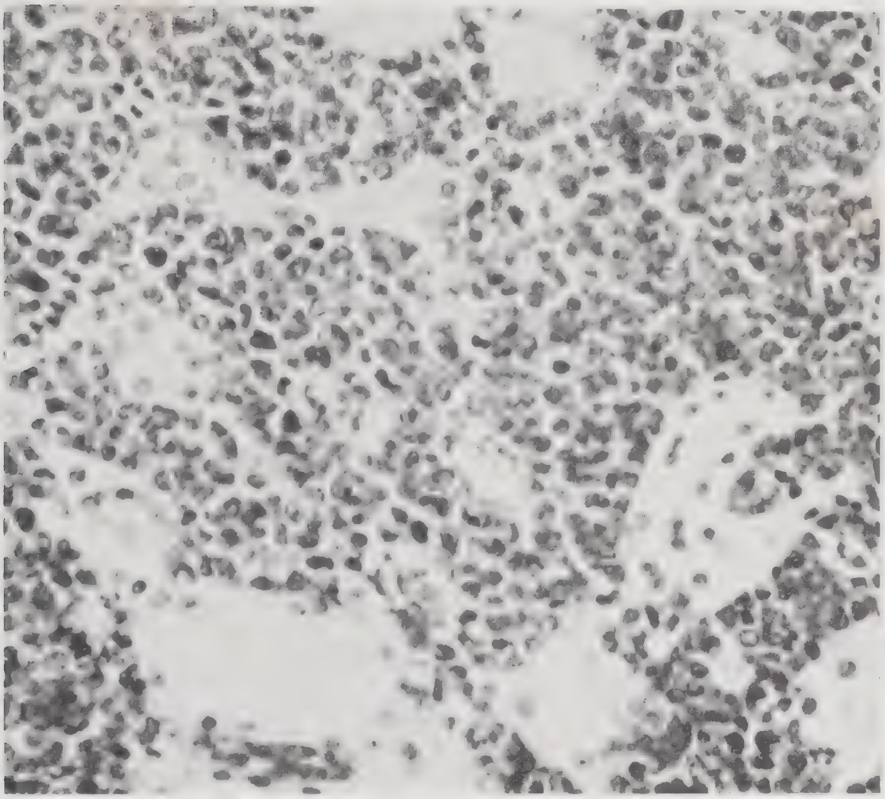

Fig. 17.14 — Leukemoid cellular accumulations.

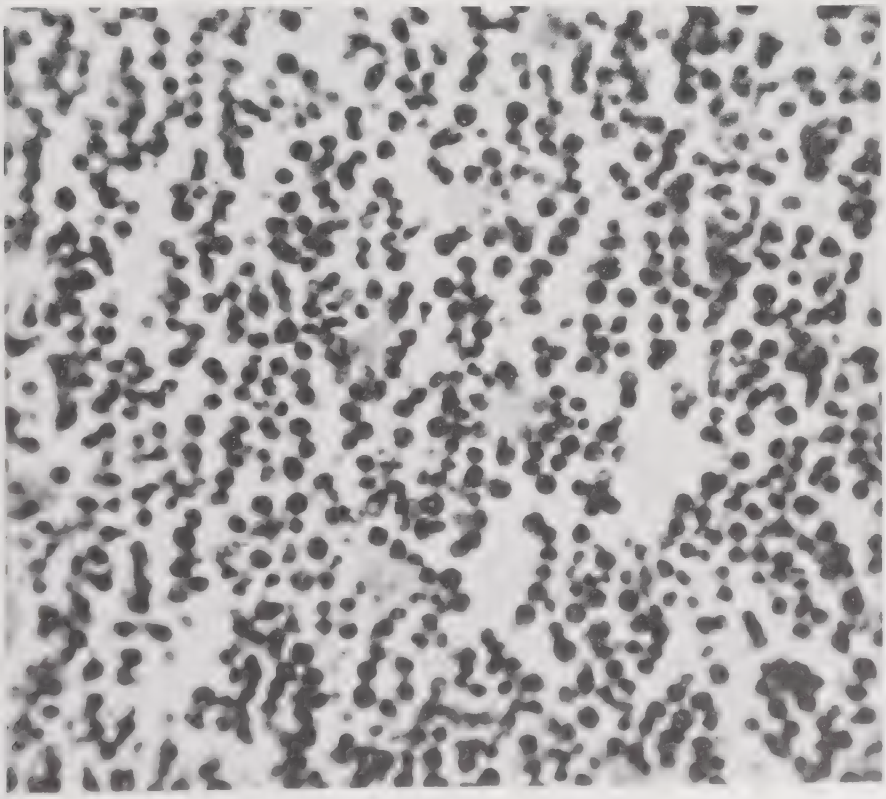

Fig. 17.13 — Leukemoid cellular accumulations.

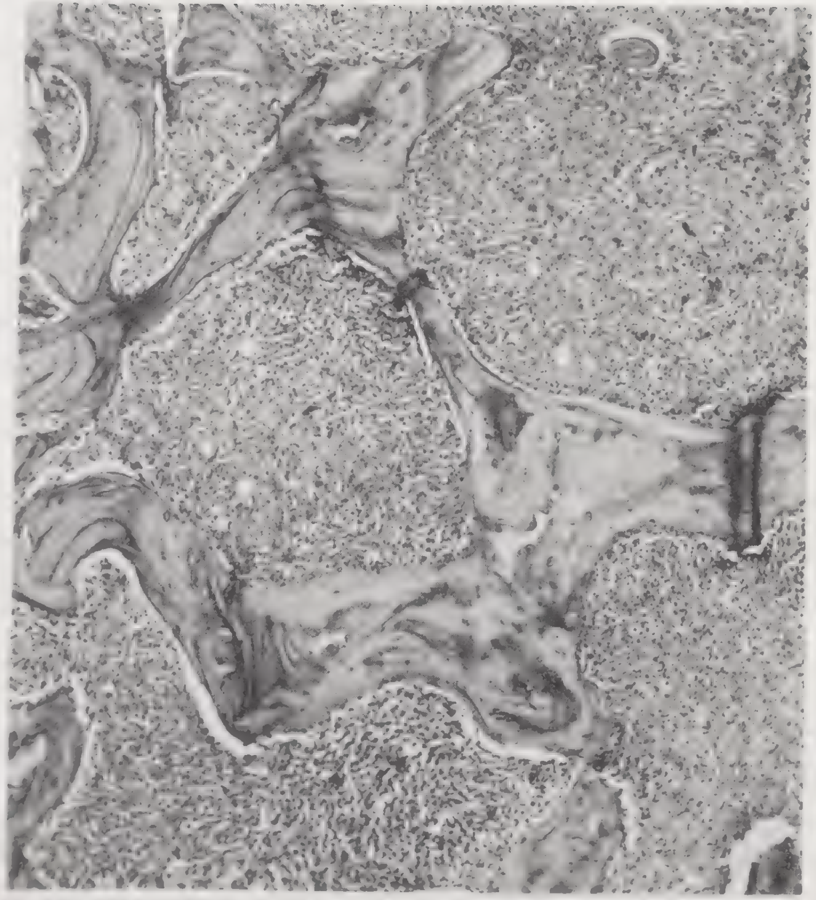

Fig. 17.15—Cellular accumulations in the bone marrow.

Fig. 17.16—Cellular accumulations in the mesentery.

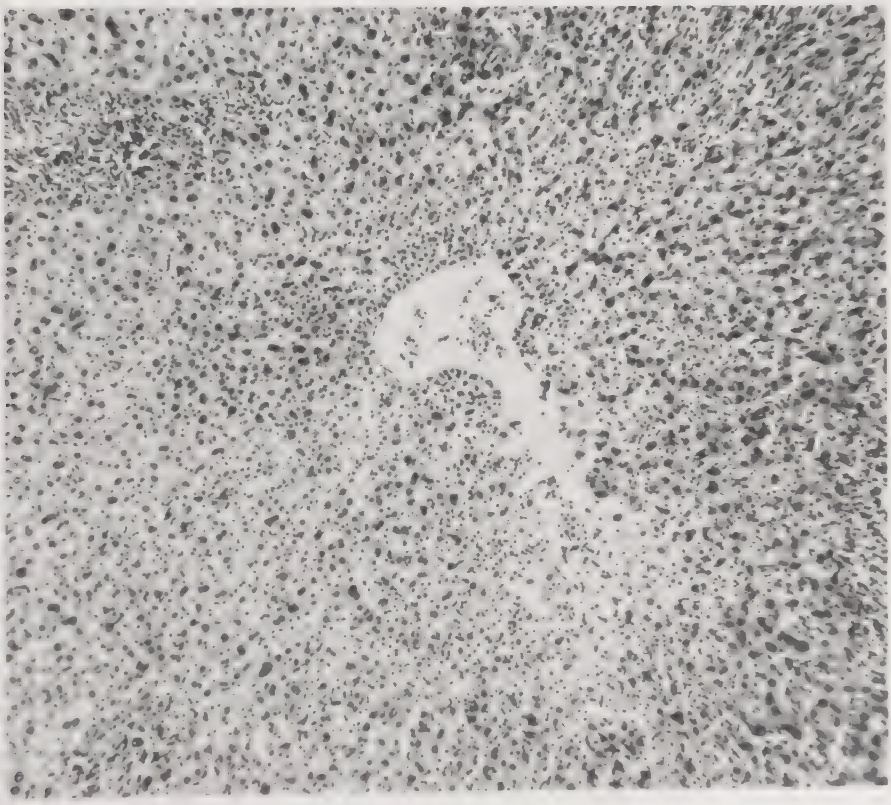

Fig. 17.17—Cellular accumulations in the liver.

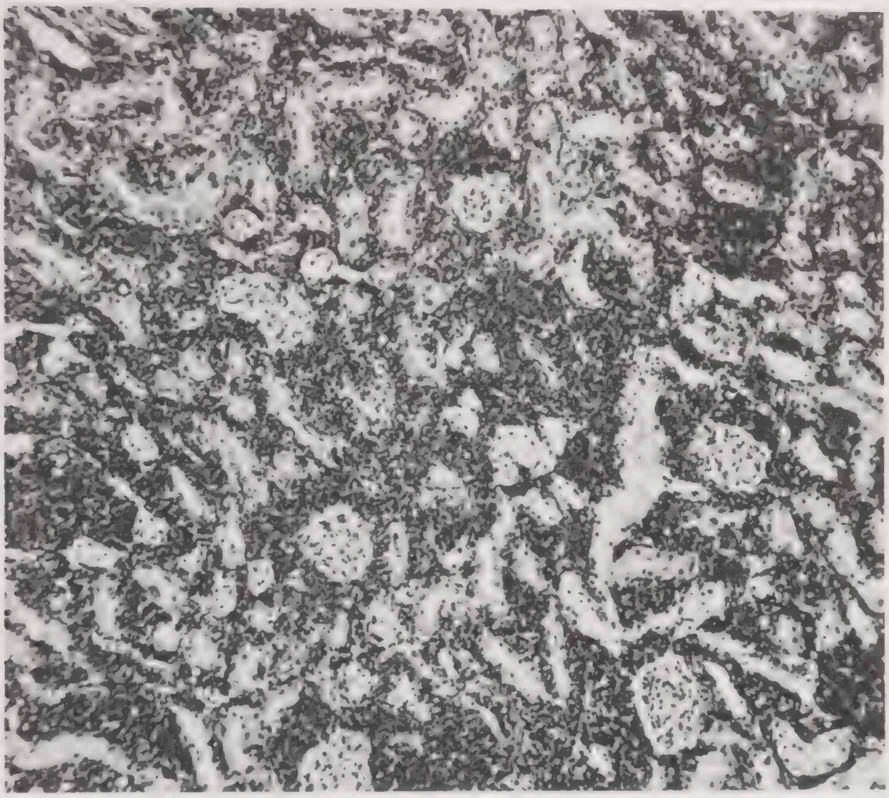

Fig. 17.18—Cellular accumulations in the kidney.

(b) Findings in Rats Sacrificed After Last Treatment. A number of rats in all groups except group 04 survived the 1-year series of exposures and were sacrificed at various time intervals as indicated in Table 17.9. The changes observed in the group killed immediately after the last treatment are presented in Table 17.15, and the results from those sacrificed at monthly intervals after the last treatment are summarized in Table 17.18. The abnormalities in the lung, bowel, and adrenal were of the same general nature as those previously described, and therefore no further description is necessary.

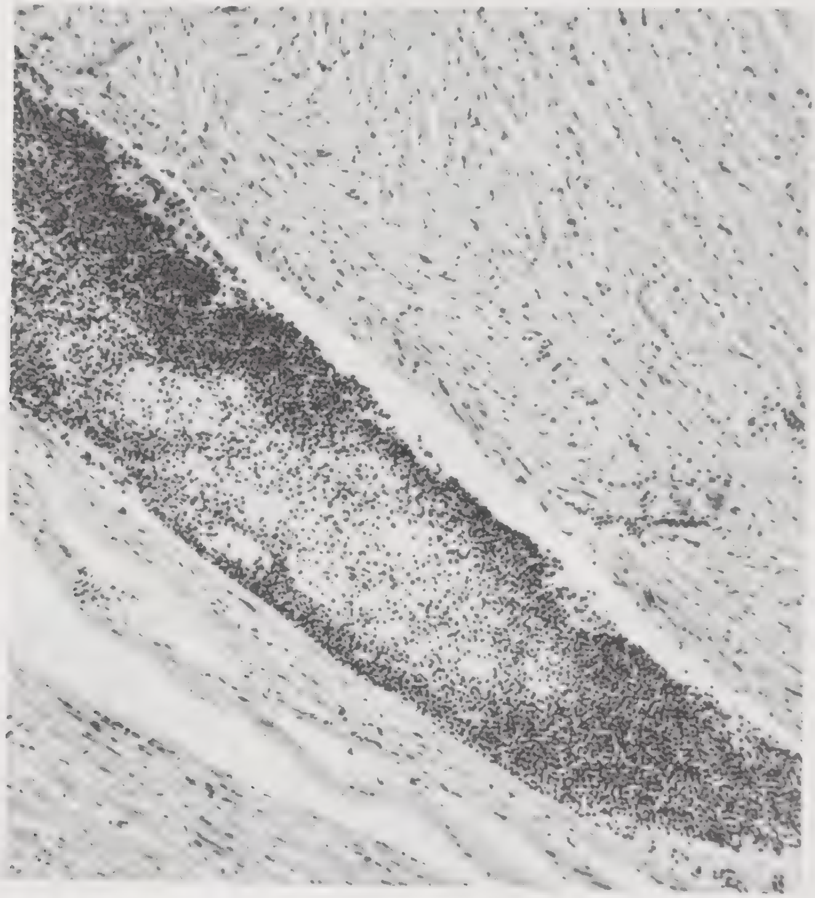

Fig. 17.19—Cellular accumulations in the heart.

Two cases were observed in which the testes showed some degree of atrophy. One of the two male rats in group 03 that were sacrificed after 2 months showed a few tubules with a reduction in the more mature germinal elements. One of four male rats in group 03, sacrificed after 3 months, showed complete depletion of germinal cells.

Table 17.17—Summary of Changes in Rats with Leukemoid Picture

Animal No.	Cellular accumulations					Predominance of young forms in marrow section	Increase in immature myeloid cells in differential count	Intravascular increase in myeloid cells in vessels and heart chambers
	Liver	Spleen	Kidney	Mesenteries	Other			
03-15	*	?				+	+	
03-28	++	++	+	+	Lung	++	++	++
04-27	++	++	+	*		++	+	+++
04-18	?	?		*		++	++	+
04-33	++	+++	++		Adrenal, lung, ovary	++		++
04-30	++	++	+	*	Lung	+	+	?
04-12	+	++	*	+	Lymph nodes, perirenal, lung, vas deferens	+		?
04-01	++	+	++	+	Seminal vesicle, epididymis	++		?
04-07	++	+	*	++		+	+	
04-08	++	+	+++	++	Lung, trachea, periadrenal, myocardium	+++	+	

*Definite but slight infiltration.

Reticulum-cell sarcomas were seen in two controls and in two of the rats in group 03 that were sacrificed immediately after the last treatment. Rat 19 of the control group showed involvement of the mesenteric nodes, whereas rat 40 showed tumor in the cervical, axillary, and mediastinal nodes, a distribution somewhat different from that commonly observed. The microscopic appearance, however, was identical with that previously described. Rat 16 in group 03 showed extensive involvement of the abdominal lymph nodes with accompanying bloody ascites. Rat 46 in this same group showed a reticulum-cell sarcoma involving the cervical, axillary, abdominal, and peritracheal nodes, which spread into the wall of the stomach and esophagus.

Table 17.18—Summary of Findings in Rats Sacrificed at Monthly Intervals After Exposure

Dosage group	Control	Control	Control	01	01	01	02	02	02	03	03	03
Time after last treatment, months	1	2	3	1	2	3	1	2	3	1	2	3
Pulmonary infection	2/6*	1/4					1/7		2/8	1/8	1/4	
Chronic ulcerative caecitis					1/4							
Hemorrhagic cysts, adrenal	1/6		1/5	2/7	1/4	4/7	2/7		3/5	1/8		2/4
Testicular atrophy												
Mild											1/2	
Severe												1/4
Malignant tumor						1/7†					2/4‡	
Benign tumor				1/7†						1/8§		

*Expressed as number of positive cases/total number examined.
†Fibroadenoma, mammary.
‡Carcinoma, mammary; fibrosarcoma, wall of cestode cyst.
§Adenoma, mammary.

A fibrosarcoma was observed in one of the four rats in group 03 (No. 42, sacrificed after 2 months). This was a large fibrosarcoma, apparently arising in the wall of a cestode cyst in the liver. This tumor showed no evidence of distant spread, although it showed histological characteristics of malignancy (Fig. 17.20).

Epithelial tumors were observed in rat 29 in group 01 and in rat 32 in group 03. The former was a papillary cystadenocarcinoma of the ovary (Fig. 17.21), and the latter was an adenocarcinoma of the breast (Fig. 17.22).

Fibroadenomata of the breast were observed in one control and in one of group 02 sacrificed immediately after the last treatment. One such tumor occurred in a rat in group 01 (No. 37) sacrificed 1 month

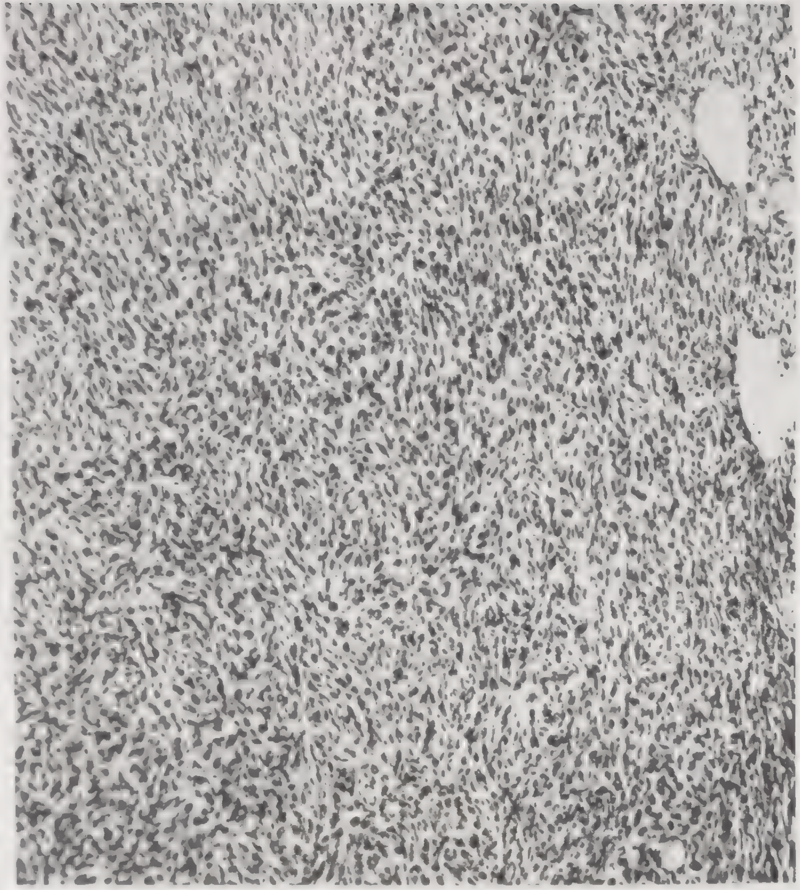

Fig. 17.20 — Fibrosarcoma in the wall of a cestode cyst.

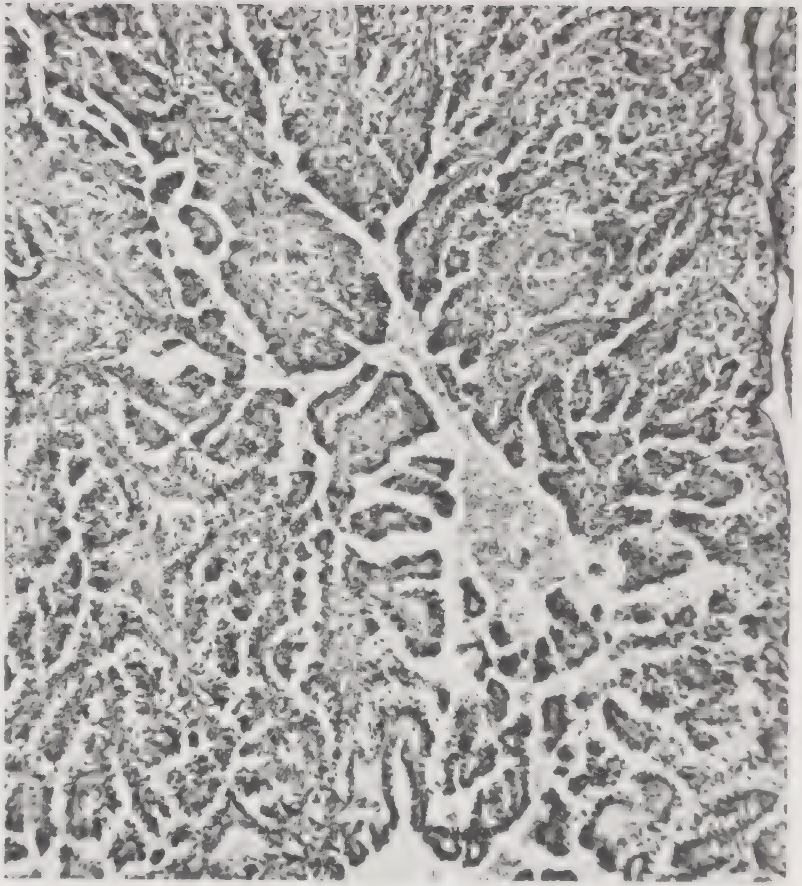

Fig. 17.21 — Papillary cystadenocarcinoma of the ovary.

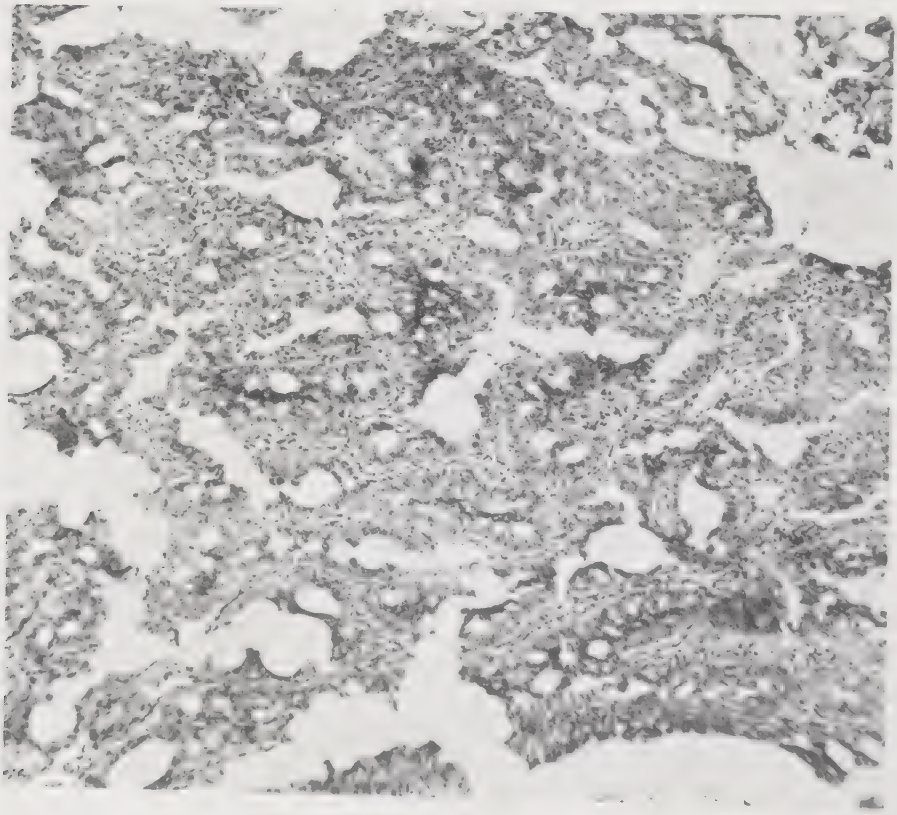

Fig. 17.22—Adenocarcinoma of the breast.

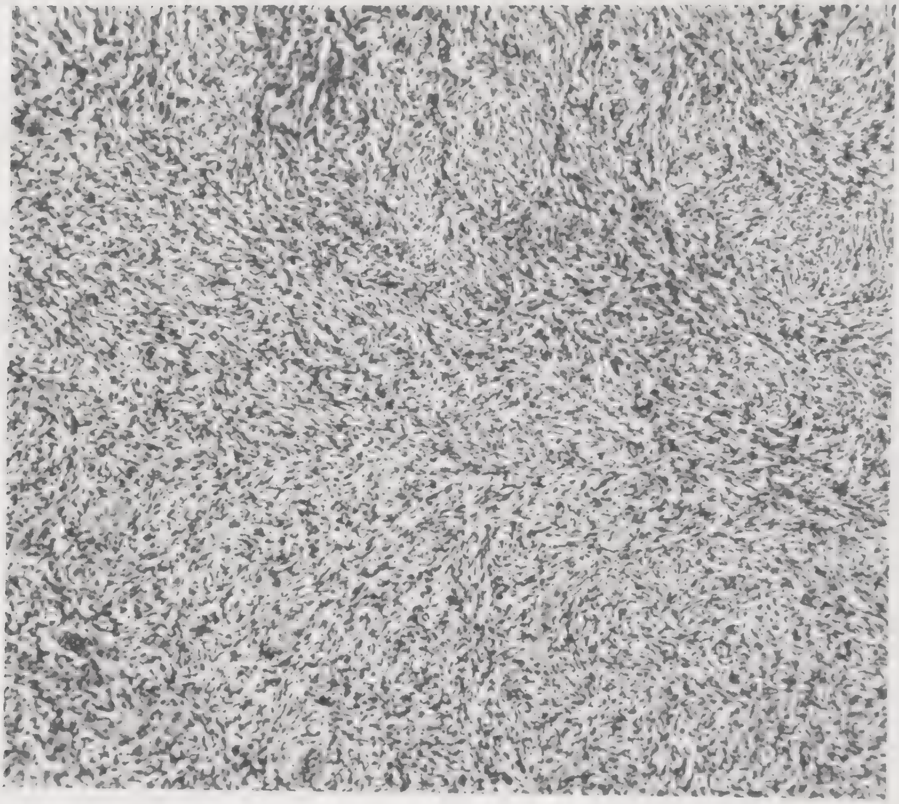

Fig. 17.23—Fibrosarcoma of the mesenteries.

after treatment was discontinued. An adenoma of the mammary gland was observed in one rat in group 03 sacrificed at this time (No. 29).

Rat 48 in the control group had a neurofibroma of the ear.

(c) Findings in Rats That Died After Experimental Period. Ten rats died in the 3-month period following the last treatment. Of the four in the control group, one showed extensive infectious involvement of the lungs, and the two females showed the hemorrhagic cysts in the adrenal which occurred so frequently in female rats in the preceding categories. No significant lesions were observed in the other rat.

Both of the rats in group 01 had malignant tumors. Rat 06 had a reticulum-cell sarcoma at the site of the caecal lymph nodes, with numerous smaller nodules in the mesenteries. Rat 05 showed very extensive involvement of the mesenteries by a fibrosarcoma (Fig. 17.23).

Of the two rats in group 02, one showed severe pulmonary edema and hemorrhage; the other showed only a mild pulmonary infection and ulceration and necrosis of the stomach wall.

Pulmonary infection was observed in the two rats in group 03, severe in one and mild in the other. The latter showed mild cystic hemorrhage in the adrenal.

3.3 Clinical Observations Pertaining to Rabbits. (a) General Condition. The rabbits of groups 00, 01, 02, and 03 showed no clinical symptoms that could be attributed to the effects of radiation. A condition, which could not be diagnosed, caused the death of five animals in group 00, three in group 01, and two in group 04. The condition was characterized by urine burns and a generally wet and dirty condition of the rear areas of the animals, a general weakness, emaciation and paralysis, and a high temperature. This condition most certainly had no relation to the effects of radiation since it occurred in the control animals as well as in the other groups. Furthermore, the condition was observed in two other animals prior to the initiation of these experiments.

Excluding the two animals of group 04 which died of this condition, none of the severe changes that occurred in the dogs of group 04 occurred in the rabbits of this group. The weight of individual rabbits of this group began to decrease about 1 month prior to death; the decrease averaged approximately 10 per cent of the maximum average weight of the animals. In only one case was emaciation marked. Usually death occurred suddenly.

(b) Weight. Figure 17.24 and Table 17.19 show that there was no significant change in the average weight of the irradiated groups in comparison to that of the control group. During the last 4 weeks of life of the individual rabbits of group 04, the weight decreased approximately 10 per cent of their maximum weight (Fig. 17.25).

(c) Mortality. Excluding the deaths of several of the rabbits due to an undiagnosed condition mentioned in Sec. 3.3a, there was no significant difference in the death rate of the rabbits of the several groups, except in the case of those in group 04. Figure 17.26 and Table 17.20 show that 70 per cent of groups 00, 01, and 03 and 100 per cent of group 02 survived the year of irradiation, whereas only 20 per cent of group 04 survived.

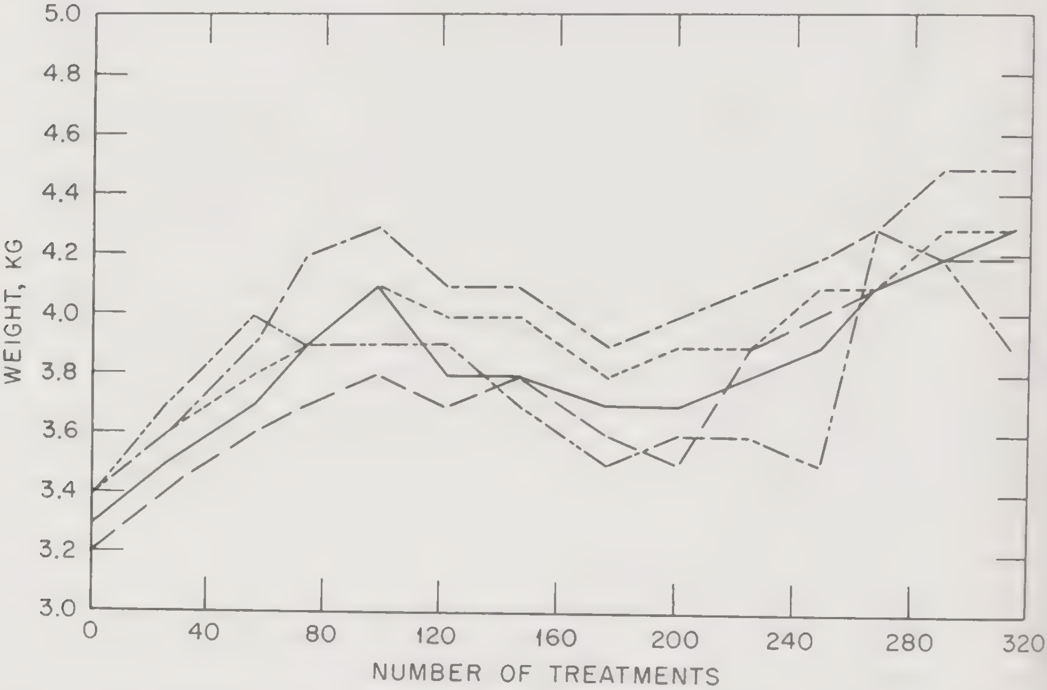

Fig. 17.24—Weights of rabbits during the period of daily irradiation with neutrons. ——, group 00. ---, group 01. ----, group 02. — —, group 03. ----, group 04.

3.4 Pathological Findings in Rabbits. As was the case with rats, it has been necessary to consider the rabbits in a number of small groups on the basis of the time of death and whether death was spontaneous or by sacrifice. Table 17.21 shows the distribution of animals in the various groups. Only male rabbits were used in this experiment.

With the possible exception of changes in the testes, there were no significant abnormalities observed in any of the rabbits in the three lower-dosage groups. Occasional cases with mild liver degeneration or necrosis and a few cases with chronic interstitial inflammation of

Table 17.19—Weight Data for Rabbits

Expt. duration, weeks	No. of treatments	Group 00			Group 01			Group 02			Group 03			Group 04		
		No. of animals	Av. wt., kg	Total dose	No. of animals	Av. wt., kg	Total dose	No. of animals	Av. wt., kg	Total dose	No. of animals	Av. wt., kg	Total dose	No. of animals	Av. wt., kg	Total dose
0	0	20	3.3	0	10	3.4	0.00	10	3.4	0.00	10	3.2	0.00	10	3.4	0.0
4	25	20	3.5	0	10	3.6	0.30	10	3.6	1.50	10	3.4	2.75	10	3.7	42.5
9	55	20	3.7	0	10	3.9	0.66	10	3.8	3.30	10	3.6	6.05	10	4.0	93.5
12	73	20	3.9	0	9	4.2	0.88	10	3.9	4.38	10	3.7	8.03	10	3.9	124.1
16	97	20	4.1	0	9	4.3	1.16	10	4.1	5.82	10	3.8	10.67	9	3.9	164.9
20	121	16	3.8	0	9	4.1	1.45	10	4.0	7.26	10	3.7	13.31	8	3.9	205.7
24	145	15	3.8	0	8	4.1	1.74	10	4.0	10.44	8	3.8	15.95	8	3.7	246.5
29	175	15	3.7	0	8	3.9	2.10	10	3.8	10.50	8	3.6	19.25	8	3.5	297.5
33	199	15	3.7	0	8	4.0	2.39	10	3.9	11.94	8	3.5	21.89	7	3.6	338.3
37	223	15	3.8	0	8	4.1	2.68	10	3.9	13.38	7	3.9	24.53	6	3.6	379.1
41	247	14	3.9	0	8	4.2	2.96	10	4.1	14.82	7	4.0	27.17	5	3.5	419.9
45	270	14	4.1	0	8	4.3	3.24	10	4.1	16.20	7	4.1	29.70	3	4.3	459.0
48	288	14	4.2	0	7	4.5	3.46	10	4.3	17.28	7	4.2	31.68	2	4.2	489.6
52	312	13	4.3	0	7	4.5	3.74	10	4.3	18.72	7	4.2	34.32	2	3.9	530.4

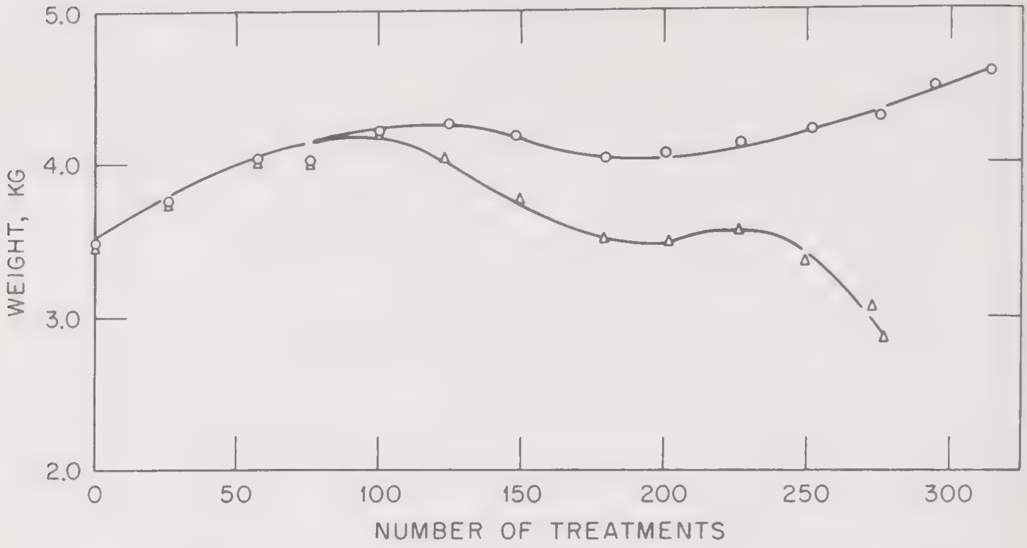

Fig. 17.25—Weight of a group 04 rabbit vs. weight of a control rabbit during the period of daily irradiation with neutrons. ○, control rabbit 6. △, group 04 rabbit 8.

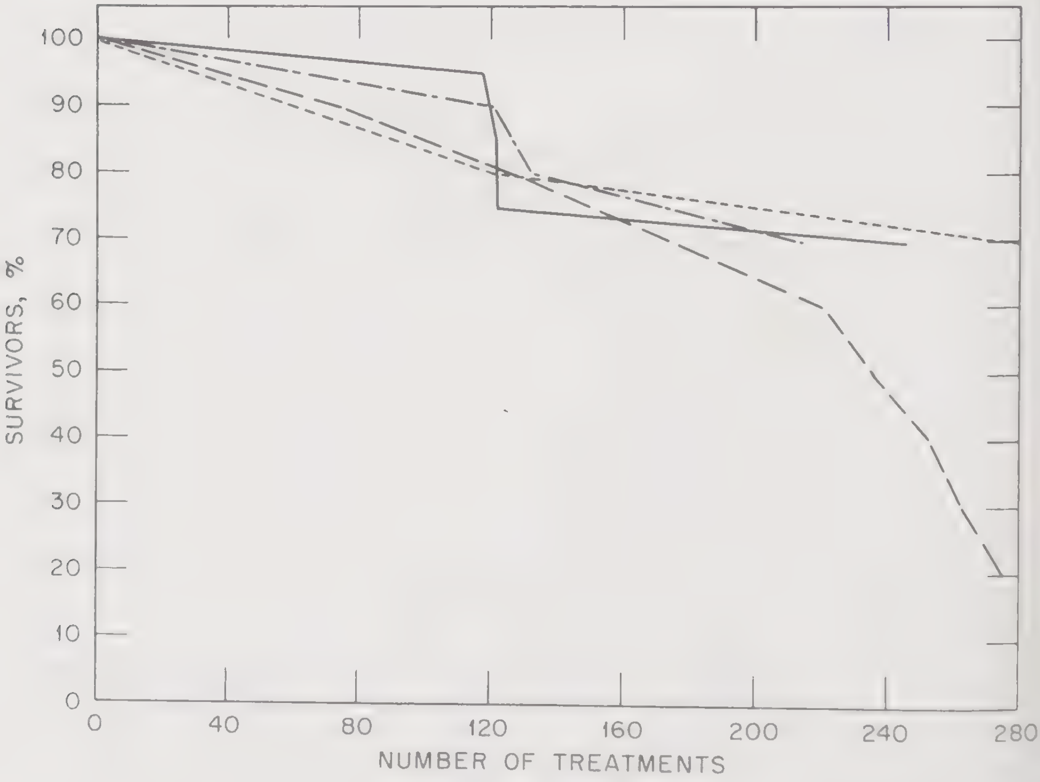

Fig. 17.26—Mortality of rabbits during the period of daily irradiation with neutrons. ——, group 00. ----, group 01. —-, group 03. — —, group 04.

Table 17.20 — Mortality Data for Rabbits

Group	Number of rabbits	Rabbit No.	Number of days in experiment to death date	Number of treatments	Total dose, n	Percentage of group surviving
00	20	15	136	117	0	95
		09	137	119	0	90
		05	140	121	0	85
		02	140	121	0	80
		18	140	121	0	75
		16	285	246	0	70
01	10	05	68	60	0.72	90
		07	140	121	1.44	80
		06	327	280	3.36	70
03	10	10	140	121	13.31	90
		04	154	132	14.52	80
		06	249	214	23.54	70
04	10	03	85	74	125.80	90
		10	145	126	214.20	80
		04	203	175	297.50	70
		07	256	221	375.70	60
		02	274	236	401.20	50
		05	295	253	430.10	40
		06	305	263	447.10	30
		08	320	275	467.50	20

Table 17.21 — Distribution of Rabbits into Groups

Group	Number of deaths during exposure	Number sacrificed at indicated time after exposure				Total
		Immediately	1 month	2 months	3 months	
Control	5	6	3	2	3	19
01	3	2	2	1	2	10
02		3	2	2	3	10
03	3	2	2	1	2	10
04	9	1				10

the kidney were seen in these rabbits as well as in the controls, but no importance is attached to them. The testes of certain rabbits in the three lower-dosage groups and in the controls were of some interest. Abnormalities of some type were observed in the testes of 2 of the 19 controls, 3 of 10 in group 01, and 3 of 10 in group 03. In one of the two controls, there was marked depletion of the germinal elements associated with a severe acute orchitis. The remainder of the cases showed some degree of cellular depletion unassociated with an inflammatory process. In general, these testes were characterized by some reduction in all germinal elements of the seminiferous tubules, with the more mature forms being most markedly affected. In most cases there was nearly complete disappearance of spermatozoa and spermatids, with only a moderate reduction in spermatocytes and spermatogonia. In addition, most cases showed the multinucleated cells which are so common in many types of testicular degeneration (Fig. 17.27). This change was mild in one case each in groups 01 and 03 and moderately marked in the remainder.

The rabbits which were exposed to 1.7 n/day not only showed a higher mortality than the other groups but also showed morphological changes in the tissues of a type commonly associated with exposure to radiation. In every case where sections of testes were available (7 of the 10 rabbits), there was complete or practically complete depletion of all germinal cells and spermatozoa. The seminiferous tubules showed only a row of Sertoli nuclei along the basement membrane and frayed pink-staining strands in the remainder of the tubular lumen (Fig. 17.28).

The bone marrow of these rabbits likewise showed very definite hypoplasia. Both the femoral and vertebral marrow were involved. In most cases the marrow cavities contained only scattered small islands of hematopoietic tissue, with the remainder of the marrow being occupied by fat (Fig. 17.29). In some instances the marrow was congested and hemorrhagic. In the case of the one rabbit which survived the entire 1-year period of exposure, the femoral epiphysis showed extreme hypoplasia of the marrow, but the vertebral marrow was more nearly normal.

With one exception the spleens from these rabbits were not considered abnormal. In one case (No. 05) there was extensive hyalinization of the splenic follicles which was negative for amyloid stain.

The type and frequency of spontaneous diseases were not significantly different in group 04 from those observed in other groups except for the occurrence of malignant tumors in two cases in group 04.

Rabbits 08 and 01, the last two of this species to die at the highest exposure level, showed involvement by malignant tumors which were very similar in their histological appearance. Rabbit 08 died after 273 treatments. In this case a small subcutaneous nodule (approximately 1.5 cm in diameter) was observed beneath the mandible; this nodule was white in color and hard in consistency. Multiple small nodules of a similar nature were also seen in the lungs. The histological appearance of the primary tumor was quite varied. Beneath the skin surface there were sheets and cords of large irregular, darkly stained cells with large, roughly oval vesicular nuclei with prominent nucleoli (Fig. 17.30). Mitotic figures were moderately

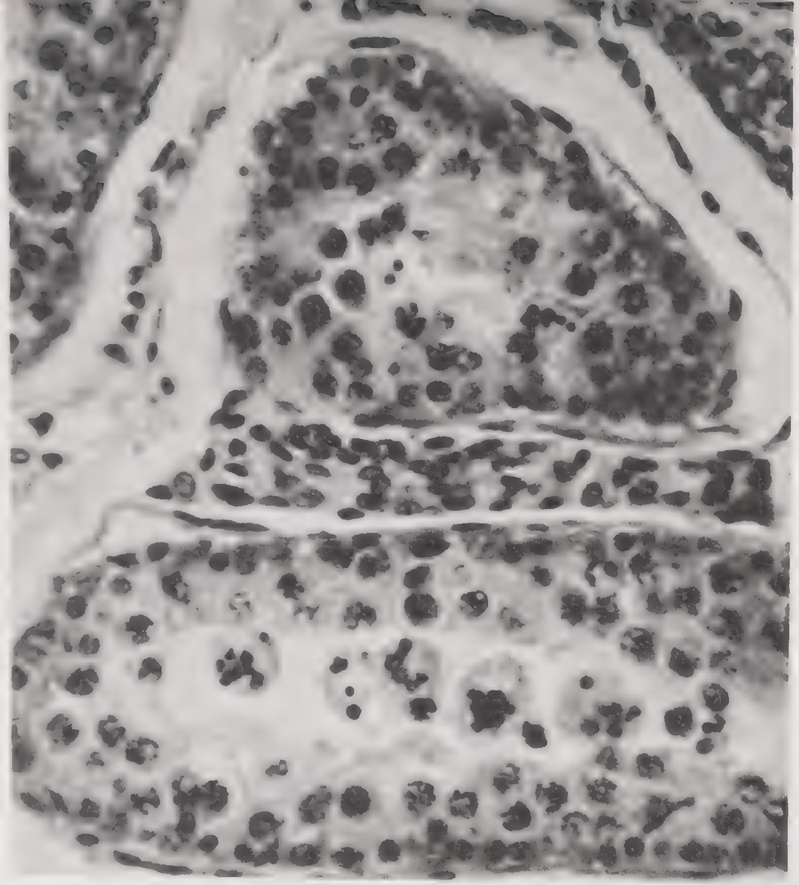

Fig. 17.27—Testicular changes in rabbits.

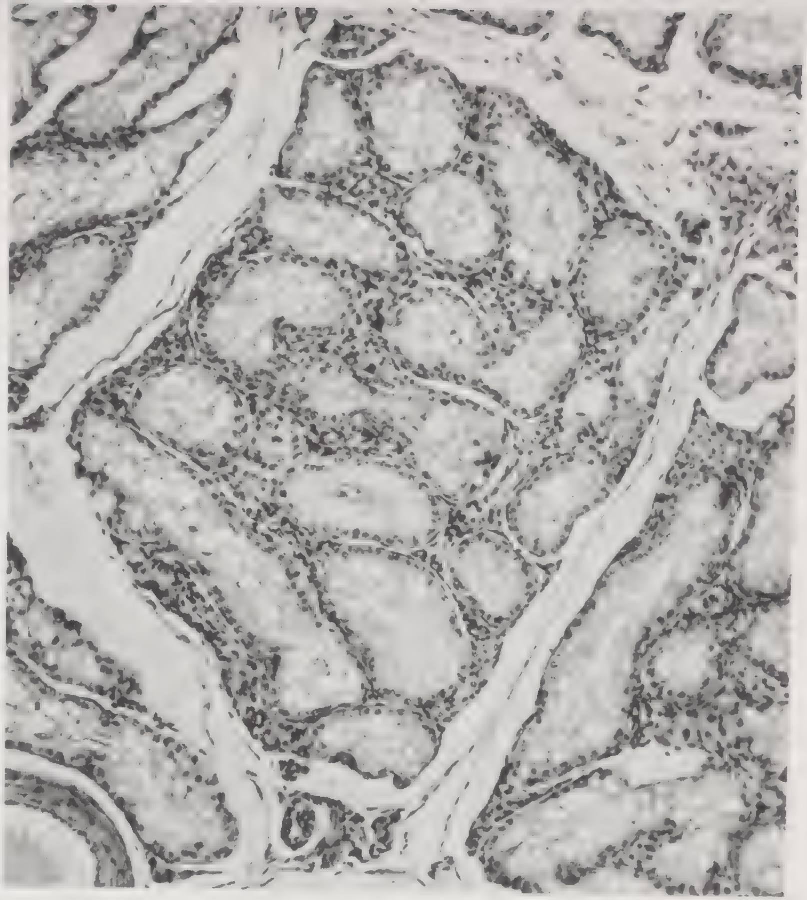

Fig. 17.28—Testicular changes in rabbits.

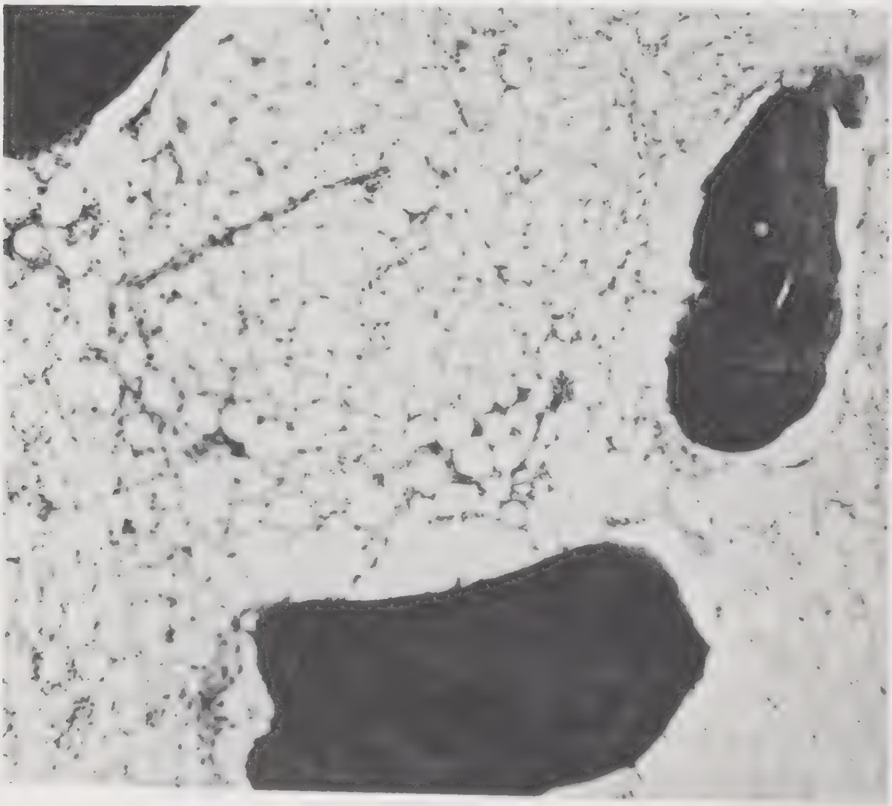

Fig. 17.29—Fatty marrow in the rabbit.

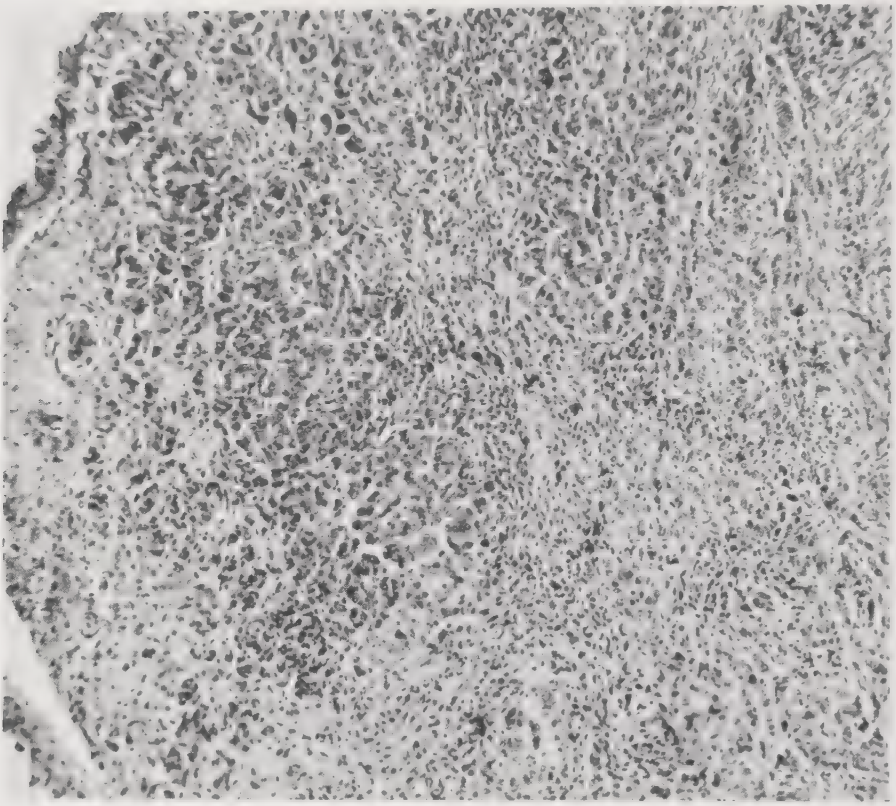

Fig. 17.30—Tumor in the rabbit.

numerous. Although most of the surface was ulcerated, in the few areas where the surface epithelium was intact there was no apparent connection between the epidermis and the well-circumscribed nests of tumor cells just beneath the epidermis. Some of these accumulations were in the form of small acinar spaces containing plugs of tumor cells. Deeper in the tumor mass the cells were somewhat smaller and paler and were separated into irregular bundles by interlacing fibrouslike strands (Fig. 17.31). In some areas these cell nests showed a tendency toward a rather ill-defined acinar type of arrangement. Some of these cell masses showed central necrosis. Still deeper in the tumor, the characteristic cells were more elongated and were arranged in interlacing bundles and whorls (Fig. 17.32). Focal areas of necrosis were scattered through this portion of the tumor. The lungs showed multiple tumor nodules varying in diameter from 1 to 3 mm. In general, the microscopic appearance of these nodules resembled the more peripheral portions of the primary tumor, i.e., large irregular cells with large oval vesicular nuclei arranged in irregular cords. In the centers of some nodules, however, there was some tendency toward more fusiform cells and arrangement in interlacing bundles (Fig. 17.33). Mitotic figures were numerous in these metastatic nodules. Some of the larger nodules showed central hemorrhage, and some showed focal necrosis similar to that seen in the primary tumor. In the kidney there was an occasional glomerular tuft plugged with tumor cells. Tumor nodules were also seen in the femoral bone marrow.

Rabbit 01 died after 311 treatments. Although no primary tumor was found, there were nodules in the lungs which were essentially identical in their histological appearance with those in rabbit 08. In the kidney there were numerous glomerular tufts plugged with tumor cells as well as one rather large tumor nodule. There were accumulations of tumor cells in the myocardium and one small nodule in the spleen.

These sections have been examined by a number of pathologists, and there is general agreement that the histological appearance and distribution of metastases as well as the site of the primary tumor suggest a diagnosis of nonpigmented melanoma. In the absence of pigmentation this possibility was not suspected at the time of autopsy; therefore special staining procedures which require fresh tissues and which might have established a definite diagnosis were not carried out.

3.5 Clinical Observations Pertaining to Dogs. (a) General Condition. During the first 5 months of irradiation, no observable clinical changes occurred in the dogs of the various groups with one exception. Dog 01 of group 04 developed during this time a severe mucoid con-

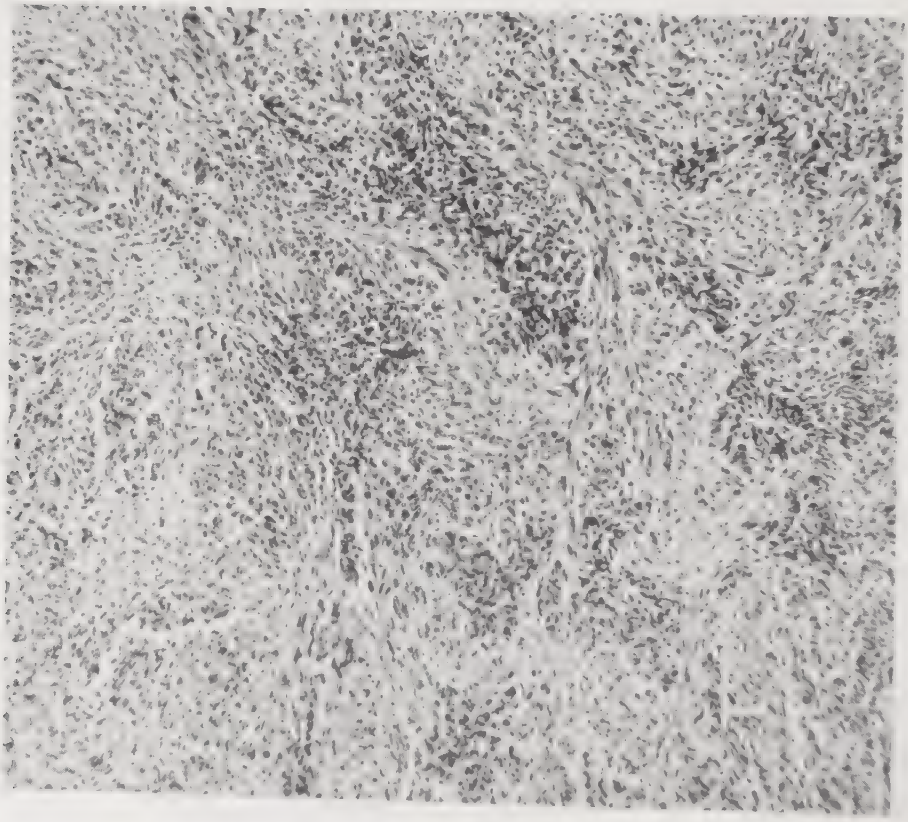

Fig. 17.31—Tumor in the rabbit.

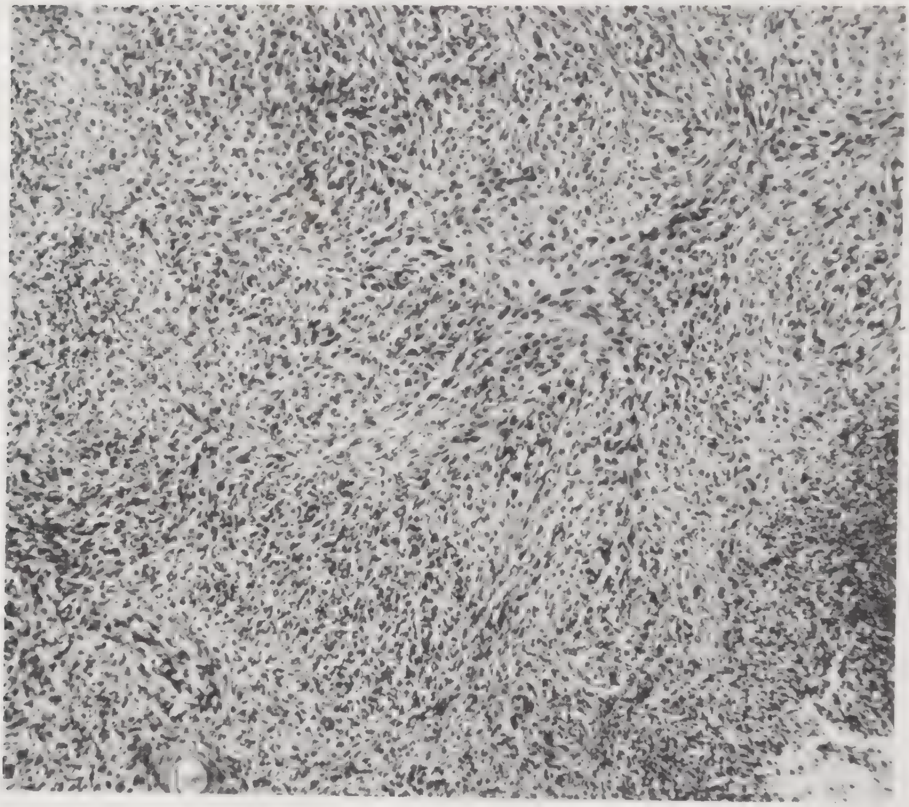

Fig. 17.32—Tumor in the rabbit.

junctivitis. This type of conjunctivitis occurred later (about 8 months from the initial irradiation) in the other dogs of group 04.

Other than the first case of conjunctivitis mentioned, loss of hair pigment was the first clinically observed change. This change occurred in all dogs of group 04 within 8 or 9 months after the beginning of irradiation but did not occur in the other groups. The graying occurred primarily at the withers, along the back, and on the shoulders to a lesser degree. The graying was diffuse in nature, i.e., not all hairs were gray in any area. The entire length of hair was involved in the loss of pigment.*

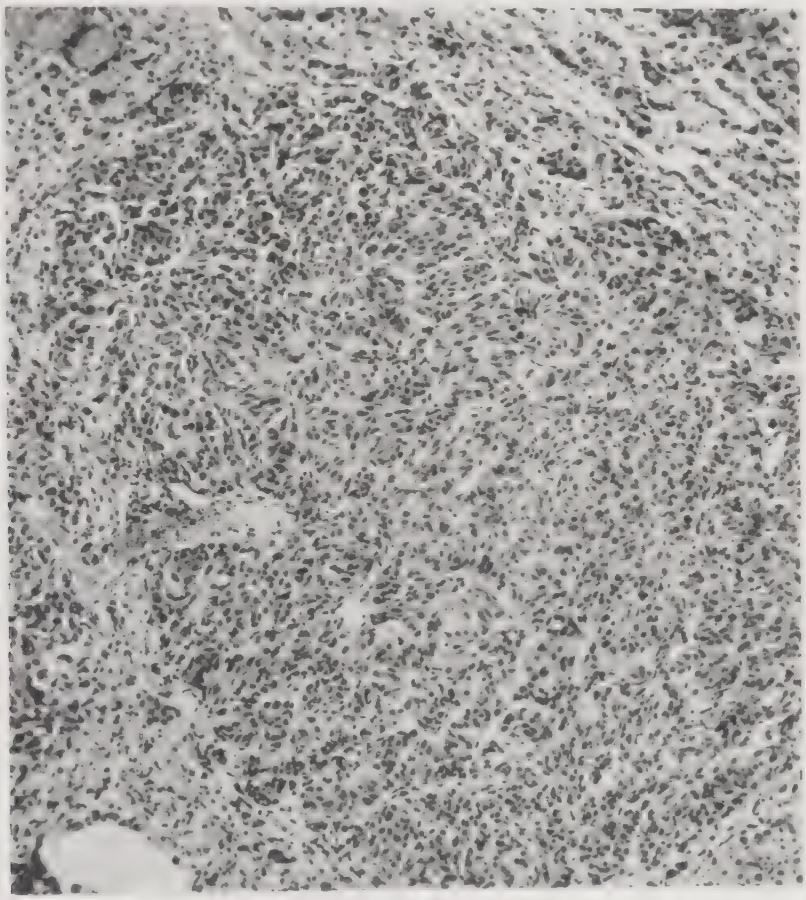

Fig. 17.33—Tumor in the rabbit.

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* The rats and rabbits used in the neutron experiments had white coats and were unsuitable for observation as to graying of hair.

All dogs of group 04 showed the same type of clinical changes, but the time of appearance of these changes varied considerably. Loss of weight occurred as early as 6 months after beginning the experiment (in dogs 02 and 05) and as late as 10 months (in dog 01). With the loss in weight the animals became lethargic. The loss of weight continued until death.

The mucoid conjunctivitis which occurred in these dogs developed into a very definite keratitis in dog 01 after approximately 11 months of treatment. There then developed a rather complete opacity of the cornea along with a loss of pigment from the iris. This sequence of events occurred earlier in the eyes of two of the other dogs of this group.

Loss of hair from the legs, ears, nose, and tail was first noticed in dog 04 (group 04) after 12 months. In the other members of this group the loss of hair occurred somewhat earlier, 9 to 11 months. At the time that the loss of hair was first noted, the ears were cold and thickened. The thickening differed from hematoma in that the ears were insensitive and cold.

A short time before death, food intake was reduced sharply, and soon thereafter the animals became progressively weaker and greatly emaciated. Dog 05 (group 04) maintained its food intake well, but its fecal material appeared to contain large amounts of undigested food.

Open wet ulcers of the skin appeared over the carpal and tarsal regions in dog 01 of this group (04) about 1 week prior to death. The surrounding tissues were edematous and greatly thickened. Dog 05 also had open ulcers similar to those of dog 01. The upper lips of dog 04 of this group were very edematous.

Electrocardiograms of dog 01 were taken 3 days before death and suggested definite myocardial damage on an anoxemic basis.

Figures 17.34 to 17.36 illustrate the physical condition of dog 01, 2 days prior to death. Figure 17.34 shows the weak emaciated condition of the dog. The condition of the forepaw is shown more clearly by Fig. 17.35. Figure 17.36 shows the much thickened left ear. The thickening of the right ear is not well shown.

(b) Weight. The data in Table 17.22 show the average weights of the dogs of the various groups at intervals during the experiment. It is apparent that there was no significant change in the average weight. Individual dogs of group 04, however, showed considerable loss of weight during the last 3 months of life (Fig. 17.37).

(c) Mortality. All dogs of groups 00, 01, 02, and 03 survived the year of irradiation. Four of the five dogs of group 04 died during the year. Dog 01 of this group was given 329 treatments, or 17 treat-

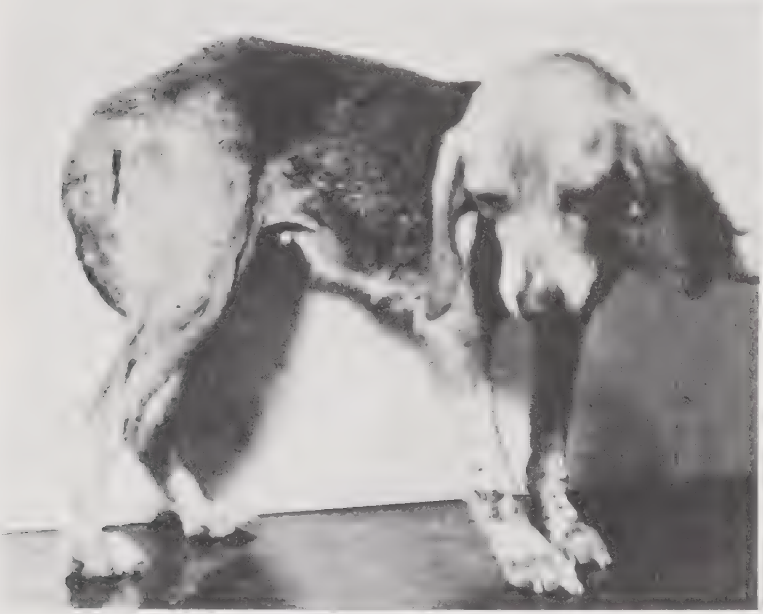

Fig. 17.34 — Photograph of dog 01 before death.

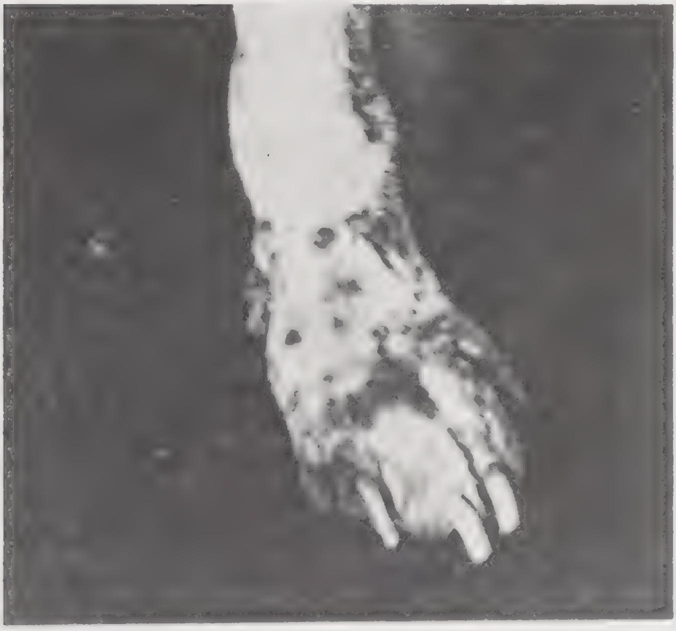

Fig. 17.35 — Photograph of the forepaw of dog 01.

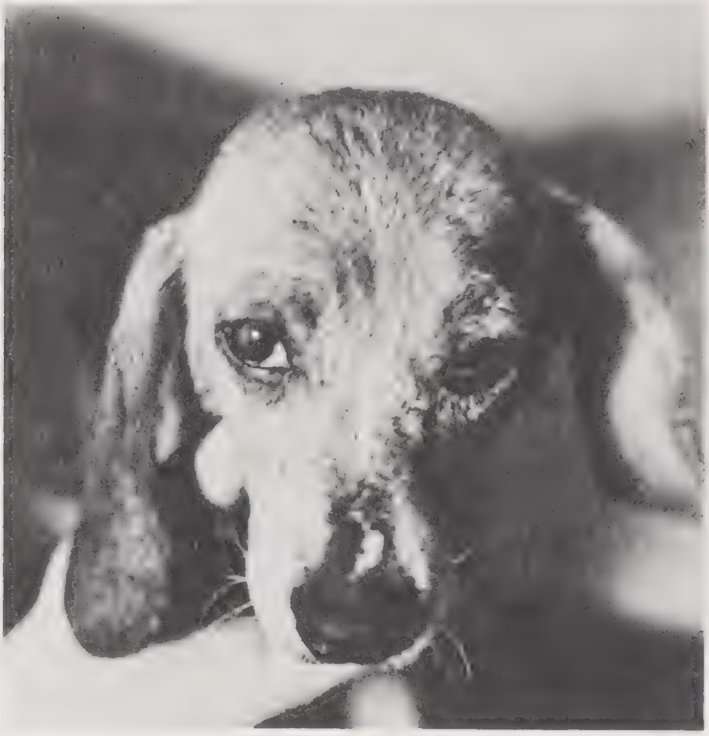

Fig. 17.36 — Photograph of the head of dog 01, showing damage to the left ear.

Table 17.22 — Weight Data for Dogs

Date	3/5/45	4/2/45	5/7/45	6/4/45	7/2/45	8/6/45	9/10/45
Number of therapy days	0	25	55	79	109	133	163
Average weight, kg							
Group 00	8.0	8.7	8.7	9.5	9.1	9.3	9.5
Group 01	8.4	9.0	9.5	9.1	9.9	9.6	9.6
Group 02	8.1	9.0	9.2	9.8	10.4	10.1	10.0
Group 03	8.2	9.1	9.1	9.8	9.6	9.9	9.7
Group 04	9.2	9.3	9.7	10.0	10.1	10.3	10.0

Date	10/8/45	11/5/45	12/3/45	1/14/46	2/4/46	3/4/46
Number of therapy days	187	211	235	270	289	312
Average weight, kg						
Group 00	9.5	9.4	9.5	9.7	9.5	9.8
Group 01	10.2	9.6	10.0	9.5	9.1	9.7
Group 02	9.9	9.7	9.8	9.0	9.1	9.5
Group 03	9.9	10.0	9.7	9.5	9.4	9.5
Group 04	9.2	8.8	9.8			

ments in addition to the year of treatment, before it died. Table 17.23 summarizes the data.

3.6 Pathological Findings in Dogs. The dogs in the three lower-dosage groups and the control dogs all survived the 1-year experimental period and were sacrificed either immediately after the last treatment or following a 3-month period in which the animals were not exposed.

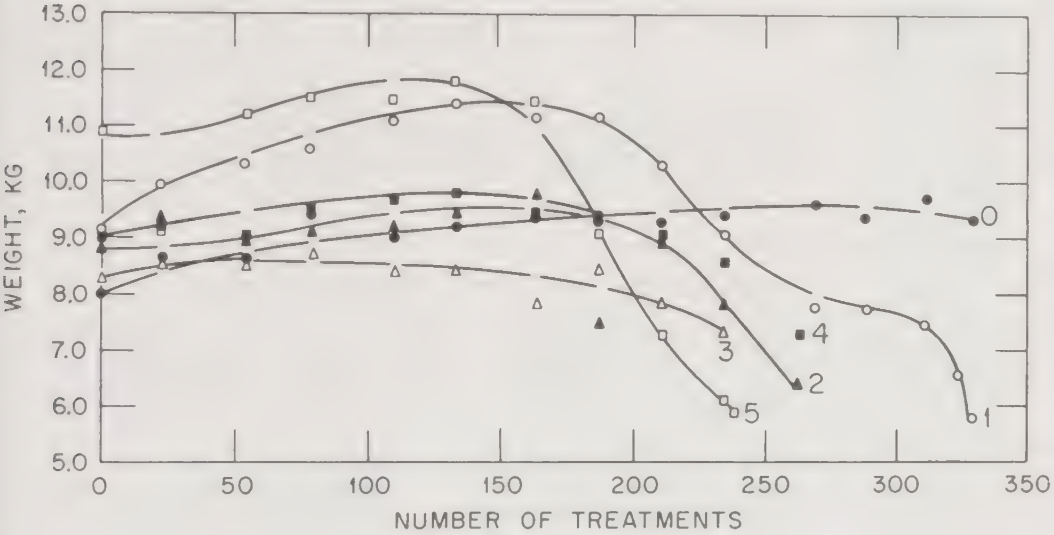

Fig. 17.37—Weights of group 04 dogs during the period of daily irradiation with neutrons. ●, control dogs. ○, dog 04-1. ▲, dog 04-2. △, dog 04-3. ■, dog 04-4. □, dog 04-5.

None of the treated animals in these groups showed changes significantly different from those observed in the controls. Of the five dogs in the 1.7 n/day group, however, four died during the period of exposure, and the remaining one died at the end of the first month post-exposure. The pathological findings in these dogs are of sufficient importance to be described in some detail.

(a) Male Dog 03 (Died After 237 Treatments). (1) Gross Findings. Two days before the death of this animal it showed a marked weakness in the hindquarters which progressed to complete prostration on the day that death occurred. A few moments prior to its death, the animal regurgitated a mixture of frothy thick fluid and undigested food. The right hind leg was edematous from the hock joint to the tarsal region. When the subcutaneous and deeper soft tissues were cut into, they were found to be hemorrhagic. There were also subcutaneous hemorrhages in the flank region. The animal's hair was coarse, rough, and grayed.

Lungs. The lungs were grayish red in color with a gelatinous appearance. There were areas over all the lobes of small old-scar formations interspersed with areas of diffuse hemorrhage.

Heart. Petechial and diffuse hemorrhages could be seen throughout the myocardium and endocardium.

Spleen. This organ had a roughened surface and a very fibrous consistency. Trabeculae were very prominent.

Pancreas. There were small petechial hemorrhages throughout the entire organ.

Liver. The liver was fibrous and mottled in color.

Adrenals. The medullary portion was very gelatinous in appearance.

Table 17.23 — Mortality Data for the Five Dogs of Group 04

Animal No.	Number of days in experiment to death date	Number of treatments	Total dose, n	Percentage of group surviving
03	275	237	399.5	80
05	276	237	399.5	60
04	308	263	447.1	40
02	309	264	448.8	20
01	384	329	559.3	0

Kidneys. The capsule was very adherent to the surface of the kidney. Throughout the cortical region there were small hemorrhages of a diffuse and petechial type, which were also observable on the surface.

Gastrointestinal Tract. The stomach demonstrated small hemorrhages in the submucosal layers varying in size up to 0.5 cm. The mucous membrane involving the entire length of the small intestine was reddened with hemorrhagic areas scattered throughout.

Testes. Hemorrhages throughout the parenchyma of the testes were noted. The testes were small and shrunken. The head of the epididymis also had diffuse hemorrhages.

Lymph Glands. These were enlarged and hemorrhagic.

Bone Marrow. The femoral bone marrow was apparently absent, the cavity being filled with fatty, colorless liquid.

Spinal Cord. There were diffuse hemorrhages over the surface in the thoracic region.

(2) Microscopic Findings. Lungs. Moderate atelectasis was present in the lungs.

Liver. A mild fatty change occurred in the liver.

Kidneys. Scattered small hemorrhagic areas in the cortex and intertubular hemorrhage were observed. (Petechiae were observed on gross examination.)

Heart. Moderate hemorrhage was found beneath the epicardium. There were also small myocardial hemorrhagic areas. (The heart was found to be hemorrhagic on gross examination, particularly in the epicardium.)

Stomach. Moderate hemorrhage occurred in the deep mucosa.

Small Intestine. Mild hemorrhage was observed in the deep mucosa and submucosa. The small lymph follicles were hypoplastic.

Large Intestine. There were no significant findings.

Lymph Nodes. Lymph sinuses were dilated and filled with blood, and there were macrophages containing blood and blood pigment. The cortex and lymph cords were thin, and no germinal centers were present. The bronchial node showed necrosis of the cortex with large clumps of bacteria.

Spleen. The capsule was wrinkled, and trabeculation was quite prominent. The follicles were slightly smaller than normal with small central accumulations of small lymphocytes and a wide peripheral zone of larger cells.

Bone Marrow. The femoral epiphysis was completely aplastic with only fat present. The vertebral marrow was markedly hypoplastic with only an occasional small island of hematopoietic activity (Fig. 17.38).

Adrenals and Pancreas. There were no significant findings in these organs.

Testes. The seminiferous tubules were small and devoid of germinal cells and spermatozoa; only Sertoli cells were present. The epididymis contained no spermatozoa. There was moderate hemorrhage in the epididymis.

(b) Female Dog 05 (Died After 237 Treatments). (1) Gross Findings. This animal was extremely emaciated, with small superficial moist irregular ulcers in the skin overlying the joints. The hair was coarse, rough, and considerably gray.

Heart. The heart was smaller than normal and of a flabby consistency.

Spleen. The spleen was very small, of a light color, and with a finely wrinkled surface. The trabeculae were prominent on the cut section.

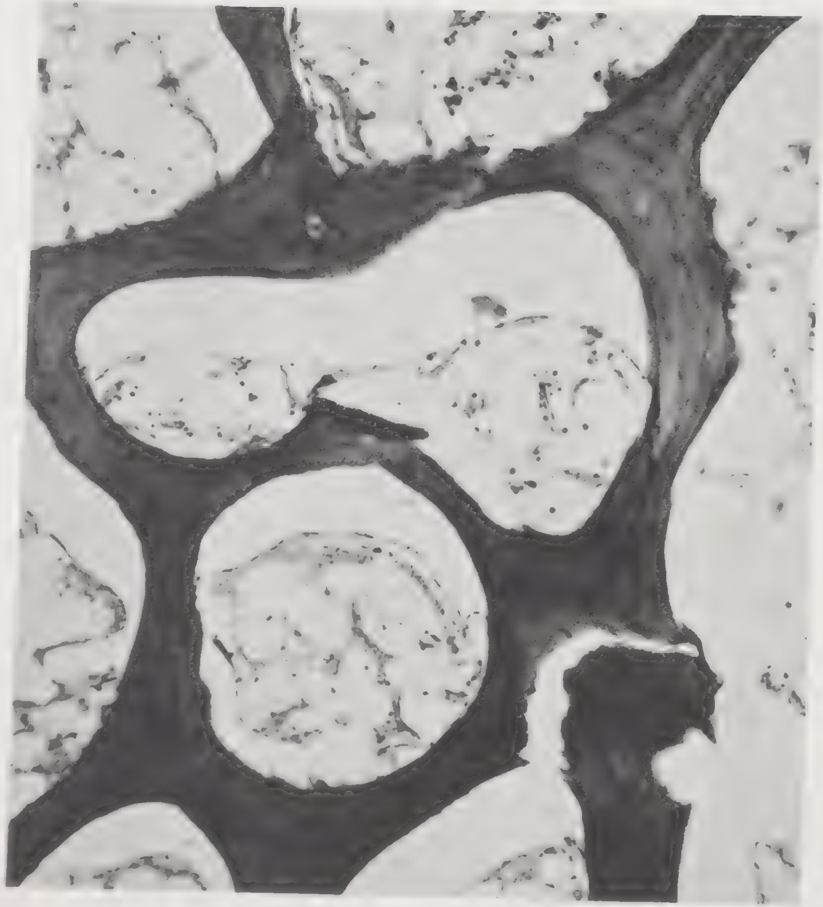

Fig. 17.38—Hypoplastic marrow of the dog.

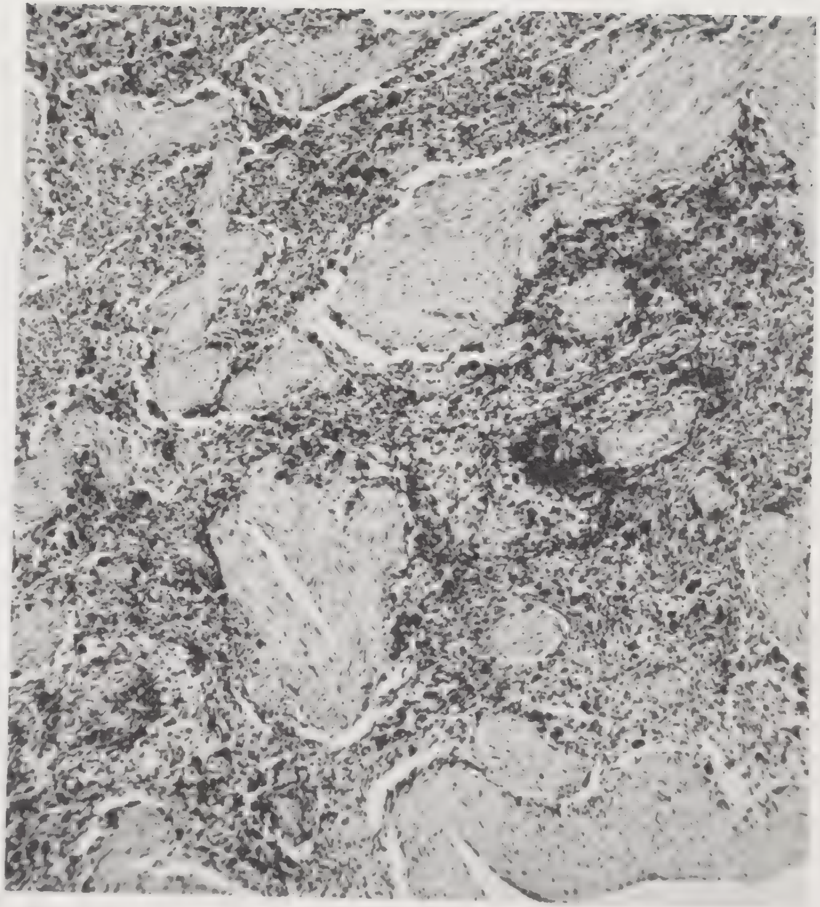

Fig. 17.39—Spleen of a group 04 dog.

Pancreas. The pancreas was soft and slimy.

Kidneys. The capsule was adherent to minute white areas on the surface of the kidney which had small pits.

Gastrointestinal Tract. Externally the stomach and intestines appeared to be normal. In the mucosa of the stomach there were scattered small petechial hemorrhagic areas. There were no such hemorrhagic areas in the large intestine.

Bone Marrow. The marrow was gelatinous and sparse.

(2) Microscopic Findings. Lungs. The lungs were essentially normal except for a small area of atelectasis.

Liver. Many sinus phagocytes containing a golden-brown globular pigment were found. The hepatic epithelium showed no significant changes.

Kidneys and Heart. There were no significant findings in these organs.

Gastrointestinal Tract. (Petechiae in the stomach and small bowel were found on gross examination.) There were no significant findings in the sections from the stomach and the small and large bowels.

Lymph Nodes. The cortex and lymph cords were very thin and hypoplastic. A tract of atrophic germinal follicles was found in one node. The sinuses were dilated and in some cases contained macrophages laden with a golden-brown pigment.

Spleen. The capsule was wrinkled, trabeculation was very prominent, and the follicles were small. Numerous pigment-filled macrophages were found in the pulp (Fig. 17.39).

Bone Marrow. Both femoral and vertebral marrow were markedly hypoplastic with scattered islands of hematopoietic activity and a few megakaryocytes. Many pigmented macrophages were found in the marrow.

Adrenals. There were no significant findings.

Ovaries. No section was available.

(c) Male Dog 04 (Died After 263 Treatments). (1) Gross Findings. All tissues of the upper lip of this dog were edematous. When cut, a diffuse hemorrhage was noted in the subcutaneous and submucosal layers. Subcutaneous hemorrhages of a very diffuse nature were found in the flank region and the muscles of the hind limbs. The entire carcass had a reddened appearance.

Lungs. The lungs were of a grayish-pink color with a wrinkled surface. There were some dark-red areas ranging up to 1.5 cm; others had a raised blisterlike center. Anthracosis was apparent. Some hard nodules were found on palpation which were not discernible on the cut section. There were some areas of a gelatinous consistency over the surfaces of the lungs. The thoracic lymph glands were hemorrhagic but normal in size.

Heart. The heart was larger than normal. Pericardial fluid was slightly blood-tinged, and petechial hemorrhages were seen over the parietal surfaces of the sac and diffuse hemorrhages over the visceral epicardium. The bicuspid and tricuspid valves were thicker than normal.

Spleen. The spleen was smaller than normal with a roughened surface. In consistency it was very fibrous, with small red areas scattered throughout.

Liver. The liver had a swollen appearance and was of an uneven color. On fresh surface the organ was pale.

Adrenals. The medullary portion was brown (probably old hemorrhage).

Kidneys. The capsule was adherent to a surface discolored with small hemorrhages and yellow areas. The cortex was mottled in color and contained small cysts. The medulla was fibrous and yellow.

Gastrointestinal Tract. The esophageal and cardiac portions of the stomach contained well-circumscribed diffuse hemorrhages in the submucosa. The small and large intestines were empty except for dark-red blood, much of it in the duodenum. Small irregular ulcers were found in the jejunum.

Testes. These organs were smaller than normal, very firm in consistency, and light brown in color. The prostate was small and fibrous.

Lymph Glands. All lymph nodes were present but hemorrhagic.

Bone Marrow. The femoral bone marrow was very sparse and fatty.

(2) Microscopic Findings. Lungs. Moderately numerous red blood cells and pigmented macrophages in alveoli were observed throughout the sections. There was one area of organizing pneumonia. Small clumps of bacteria were evident.

Liver. A mild fatty change occurred in this organ.

Kidneys. Several small areas of intertubular hemorrhage appeared in the outer cortex and also several small intertubular clumps of bacteria.

Heart. (Hemopericardial and subepicardial hemorrhage were observed grossly.) No microscopic evidence of hemorrhage was found in the section examined.

Gastrointestinal Tract. (Hemorrhage was seen on gross examination.) There were no significant findings in the sections of stomach, small bowel, and large bowel.

Lymph Nodes. A moderate to marked thinning of the cortex and lymph cords and an occasional atrophic follicle were seen. The sections of all nodes showed dilated sinuses filled with red blood cells and macrophages containing red cells and blood pigment.

Spleen. The follicles were only slightly smaller than normal. Trabeculation was prominent, and the capsule was only slightly wrinkled. Small clumps of bacteria were discerned.

Bone Marrow. The femoral epiphysis showed almost complete aplasia; only fat was present. The vertebral marrow was only moderately hypoplastic with the vessels engorged.

Testes. The seminiferous tubules were small and devoid of germinal cells; only Sertoli cells were present, and no spermatozoa were seen in the epididymis (Fig. 17.40).

Skeletal Muscle. (Hemorrhage was observed grossly in the musculature of the legs.) The section of the skeletal muscle showed extensive hemorrhage.

(d) Male Dog 02 (Died After 264 Treatments). (1) Gross Findings. This dog was very emaciated and dehydrated. The hair was rough, coarse, and considerably gray. The musculature was reddened.

Lungs. The lungs had a gray cast and contained many circumscribed areas with deep-red gelatinous centers. Many minute hard nodules were felt throughout the substance of the lung which were not discernible on fresh section. The trachiobronchial lymph nodes were hemorrhagic, dark, and small.

Heart. This organ was flabby and relatively empty of blood. There were small myocardial hemorrhages, especially in the apex.

Spleen. The spleen was very small and fibrous.

Pancreas. The pancreas was normal in size and shape but pale in color and of a slimy consistency.

Liver. The liver was hard and fibrous.

Kidneys. The capsule was adherent. The surface of the kidney was rough, pitted, and speckled with dark-red spots. Petechial hemorrhages were present in the substance of the cortical region.

Gastrointestinal Tract. There was petechial hemorrhage in the submucosal layers of the stomach with digested black blood in the lower portion of the small intestine and in the duodenum.

Testes. The testes were dense in consistency and yellow brown on cut section. There were diffuse hemorrhages over the surface of the seminal vesicles.

Lymph Glands. All were enlarged and hemorrhagic.

Bone Marrow. The femoral bone marrow was sparse, fatty, and bright red.

(2) Microscopic Findings. Lungs and Liver. There were no significant changes in these organs.

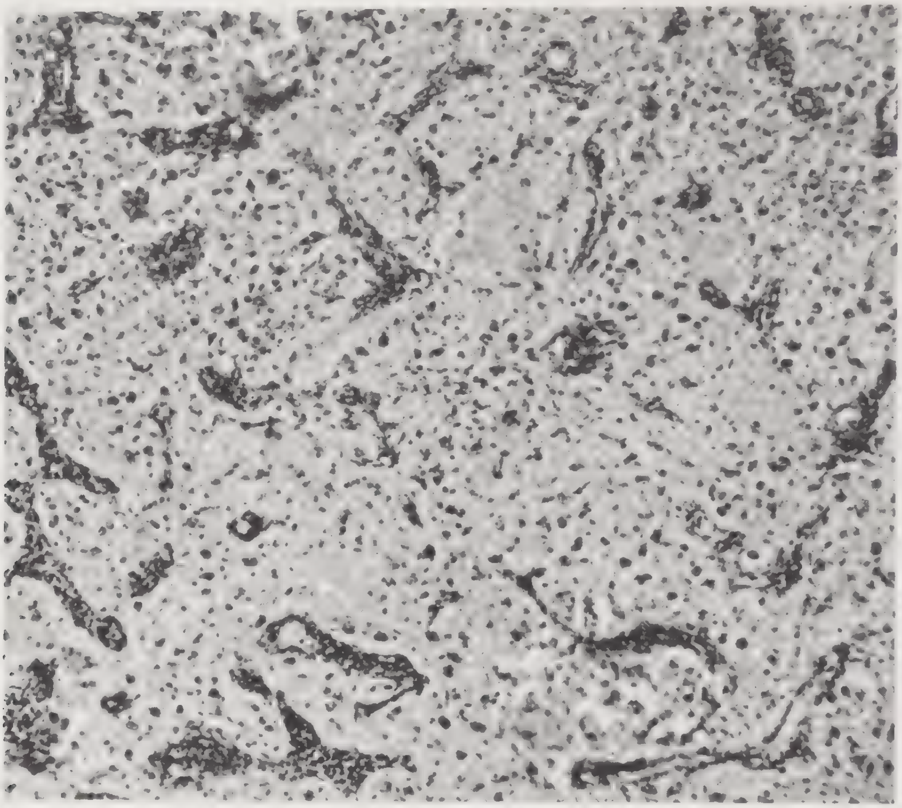

Fig. 17.41 — Lymph node of a group 04 dog.

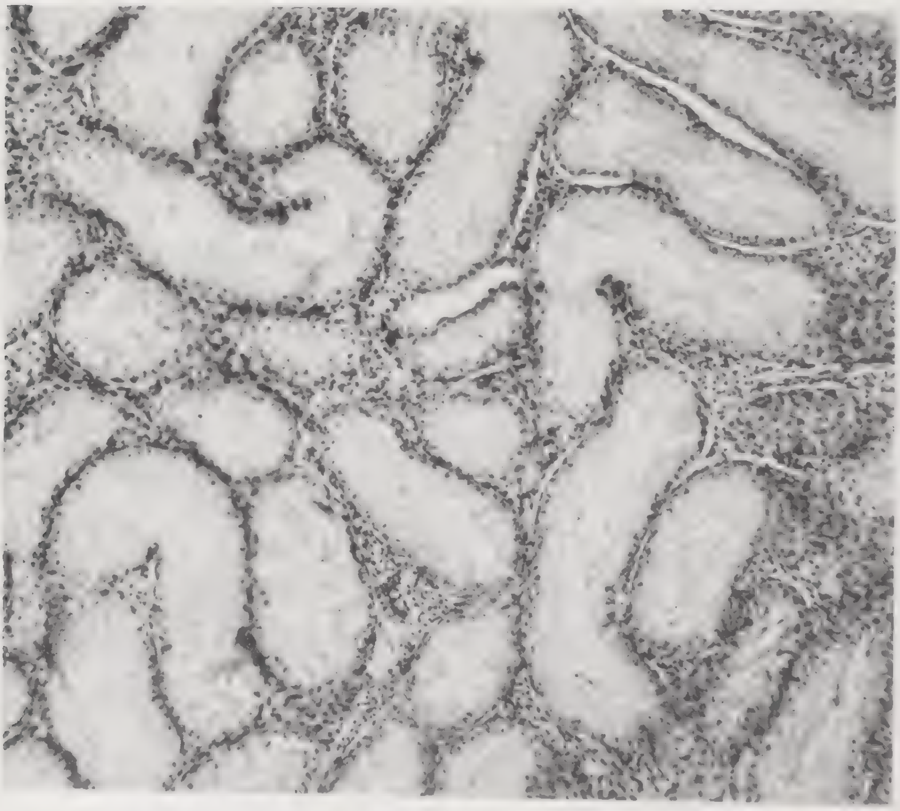

Fig. 17.40 — Testes of a group 04 dog.

Kidneys. A few small scarred areas were observed in the outer cortex.

Heart. (Hemorrhages in the heart were observed grossly.) There was no evidence of hemorrhage in the section examined microscopically.

Gastrointestinal Tract. There were no significant changes in the sections of the stomach and large intestine. The area of hemorrhage was in the mucosa of the duodenum.

Lymph Nodes. The lymph cords and cortex were narrow and hypoplastic; a few small germinal centers were discernible in one section of the bronchial node. Sinuses in all the nodes were dilated and contained blood and blood-filled macrophages (Fig. 17.41).

Spleen. The follicles were small, trabeculae were prominent, and the capsule was moderately wrinkled.

Bone Marrow. Marrow of the femoral epiphysis showed marked hypoplasia. The femoral shaft showed several large islands of densely packed marrow cells, and the vertebral marrow was moderately hypoplastic.

Adrenals. There were no significant findings.

Ovaries. Only primordial and small growing follicles were present; many of these were degenerate (Fig. 17.42).

Eyes. Hemorrhage occurred at the sclerocorneal junction in the pectinate ligament, and the anterior chamber was filled with blood.

3.7 Effect of Neutron Radiation on Testicle Size. The testes of the dogs, rabbits, and rats of group 04 were considerably reduced in size, as observed at autopsy. Weight data, however, were collected only on the organs of the dogs. The data in Table 17.24 show the change in the size of the testes of these dogs. Since the size of the testes would be expected normally to vary with the size of the animal, the relative size of the testes in relation to the size of the dogs of each group is taken into consideration in computing the data.

It may be seen in Table 17.24 that the testes of group 04 were greatly decreased in size in relation to the animal weight. Those of group 03 were also decreased somewhat in size.

3.8 Effects of Neutron Radiation on the Cellular Elements of the Peripheral Blood. Owing to the extreme variability of counts of the cellular elements of the peripheral blood in the rats, rabbits, and dogs, the results of the hematological studies were difficult to interpret. In most instances the trends, if present, could be described only in a general way.

For the most part, only group 04 showed significant trends of any of the cellular elements of the peripheral blood. Consequently the lower-dosage groups are mentioned only when the occasion merits.

(a) Lymphocyte Trends. (1) Rats. The lymphocyte counts decreased rapidly during the first month of exposure to 1.7 n/day. Thereafter the depression progressed very little, and, although the counts varied considerably, they were consistently lower than the counts of the controls and the three lower-dosage groups. Statistical interpretation of the results was difficult because of the wide variation of counts within each group. However, the depression in group 04 is considered to be real.

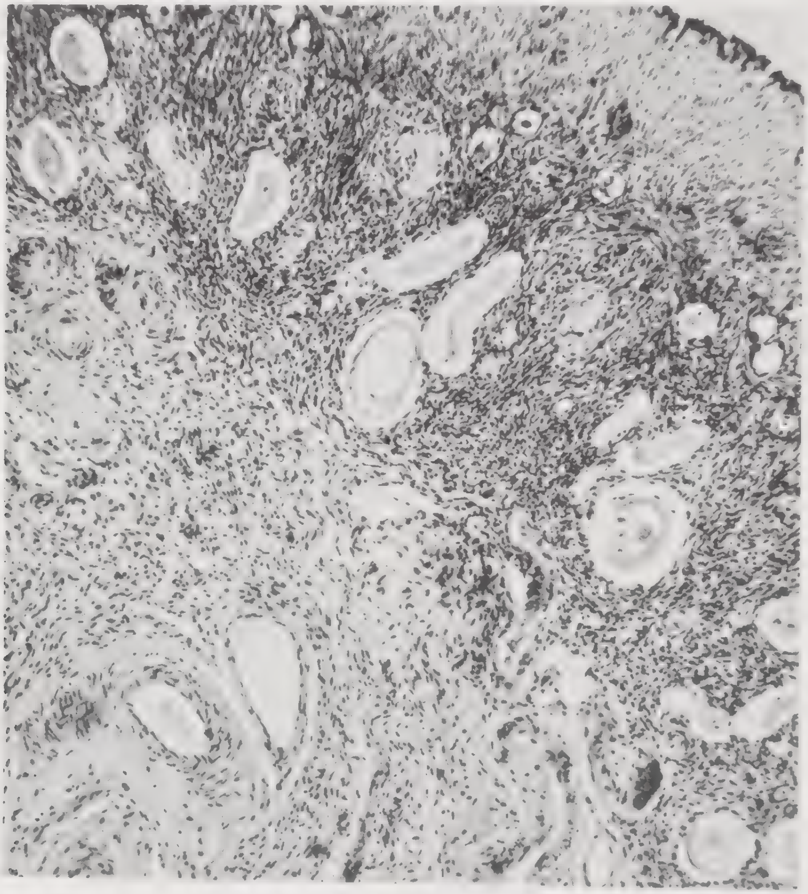

Fig. 17.42—Ovary of a group 04 dog.

(2) Rabbits. Lymphocyte counts of rabbits exposed to 1.7 n/day fell progressively after the beginning of exposure, and at the end of the experiment they were approximately 30 per cent of the preexposure level. This trend diverged markedly from the trends of the lower-dosage groups and controls, all of which showed no significant change during the course of the entire experiment.

(3) Dogs. Lymphocytes of group 04 dogs were definitely decreased after approximately 4½ weeks of exposure. The trend of the counts continued steadily downward, and, when the experiment ended after 54 weeks, the count of the single survivor was approximately 9 per cent of the preexposure mean count.

There appeared to be a slight depression of lymphocytes in all other treatment groups also, but these trends were not statistically significant.

Table 17.24 — Relative Size of Testes of the Dogs at Autopsy

Group	Number of dogs	Average number of treatments	Average weight of dogs, kg	Average weight of testes, g	Animal weight / Testicle weight	Percentage change in testicle
00	3	312	11.4	11.0	1036.4	
01	3	312	10.5	12.0	875.0	+15.6
02	3	312	10.6	10.6	1000.0	+3.5
03	3	312	9.1	8.0	1137.5	−8.9
04	3	254.7	6.9	4.4	1568.2	−51.4

(b) Neutrophil Trends. (1) Rats. There was considerable fluctuation in neutrophil counts of all treatment groups throughout the experiment. During the first 17 weeks of exposure the neutrophils of group 04 appeared to be slightly depressed in relation to the other groups. The depression was not statistically significant, however. After this slight preliminary depression the trends of all groups were distinctly upward, and the trend of group 04 became indistinguishable from the trends of the lower-dosage groups and controls. At the end of the experiment, counts in all groups were definitely above normal levels.

(2) Rabbits. Absolute neutrophil counts were extremely variable in all groups. After approximately 8 weeks of exposure, however, counts of group 04 were both apparently and statistically lower than counts in the other groups. This difference was maintained throughout the remainder of the experiment.

(3) Dogs. Neutrophils of dogs exposed to 1.7 n/day were depressed within 2 weeks after the beginning of exposure. Although the depression is not statistically significant, it is apparently real because, with the exception of a few irregularities, the trend continued downward and diverged markedly from the trends of the controls and the three lower-dosage groups throughout the remainder of the experiment.

(c) Trends of Total Leucocytes. (1) Rats. In rats the variation in the total leucocyte count was extreme. The over-all trend first reflected the early marked depression in both lymphocytes and neutrophils. During the latter part of the experiment, however, when neutrophils were composing an increasing percentage of the total leucocytes, the total leucocyte trend tended to parallel the markedly variable, rising neutrophil trend.

(2) Rabbits and Dogs. The leucocyte trends in rabbits and dogs treated with 1.7 n/day reflect the trends of both the neutrophils and lymphocytes. In general, the trends were steadily downward and were divergent from trends of the other treatment groups and controls.

(d) Platelet Trends. (1) Rats and Rabbits. Extreme variability characterized the platelet counts in all treatment groups of rats and rabbits. No statistically significant or readily apparent trends were noted in any group.

(2) Dogs. The platelet counts in all groups of dogs were extremely variable, and no statistically significant trend was noted. By inspection, the platelet counts appear to be somewhat lower in group 04 after approximately 25 weeks of treatment, but this trend is not striking.

(e) Erythrocyte Trends. (1) Rats. Owing to the marked variation in erythrocyte counts, particularly in the control group, statistically significant trends of the erythrocytes of group 04 rats were not demonstrable. After approximately 32 weeks, however, an apparently definite downward trend of erythrocytes began and persisted throughout the experiment.

(2) Rabbits. Erythrocyte counts of all rabbits showed wide fluctuations throughout the experimental period. After approximately the thirty-fourth week, however, erythrocytes of group 04 were definitely depressed relative to the counts in the control and the lower-dosage groups. The depression persisted, with marked variations in counts, throughout the remainder of the experimental period. Because of the great variations the extent of the depression cannot be described exactly.

(3) Dogs. After approximately 35 weeks of irradiation with neutrons, erythrocytes of group 04 dogs fell to approximately 80 per cent of their preirradiation level. Only one animal survived beyond the forty-third week, and its erythrocyte count ranged from approximately 70 to 80 per cent of the preirradiation level until the last 2 weeks of exposure (fifty-third and fifty-fourth), at which time it dropped still further. During the last week the survivor's count was only 40 per cent of the preexposure level.

(f) Hemoglobin Trends. In all cases the hemoglobin trends were very similar to the erythrocyte trends.

(g) Reticulocyte Trends. No significant trends were observed in the reticulocyte counts of any of the treatment groups. In all cases the counts were extremely variable.

(h) Summary of Hematological Findings. The significant findings in the hematological investigation may be summarized as follows:

1. Lymphocytes showed the greatest response to neutron irradiation and, in dogs, appeared to have been slightly depressed even in the three lower-dosage groups.

2. Neutrophils were depressed within 2 weeks after exposure to 1.7 n/day in dogs and after 8 weeks of the same exposure in rabbits. The apparent increase in neutrophils in the same treatment group in rats is probably an artifact which reflects the tendency of these animals to develop infections and severe leucocytoses in long-term experiments. A similar phenomenon, which was observed in the chronic X-ray studies, was responsible for the exclusion of the rat data from Chap. 11.

3. Erythrocyte depressions were the last to occur and were noted in dogs, rabbits, and rats after 32 to 35 weeks of irradiation with 1.7 n/day.

4. DISCUSSION

4.1 Clinical Studies. It seems apparent that the amount of irradiation administered to groups 01, 02, and 03 produced no observable changes in rabbits and dogs. In rats the incidence of internal tumors in these groups was sufficiently increased over the incidence in group 00, the control group, to justify the assumption that the neutron radiation was a contributing factor in their origin. The mortality of the rats in group 03 appears to have been increased during the last one-third of the experiment.

The amount of irradiation given to group 04 of each species of animal used produced profound effects. The survival time of these animals was greatly reduced, and their general health and physical condition were adversely affected. Although all animals in group 04 (except two rabbits which were killed and one dog which survived) died during the year of irradiation, approximately two-thirds of the rats and rabbits and all the dogs in the other groups survived the year and were in good condition. The profound effects of the dosage administered to the animals in group 04 are emphasized also by the cataracts produced in rats, the graying of hair in dogs, the loss of weight in all animals, the reduction of food intake, and general debilitation.

Lenticular opacities occurred relatively late in the rats and might have had a higher incidence if the number of animals surviving after

200 treatments had been greater. It is difficult to suggest the nature of these opacities, but it is interesting to note that there was a marked similarity between the cataracts in group 04 rats and that their absence in the other groups eliminates the possibility of a chronological age factor accounting for this high incidence. In only one other animal (group 01) did a cataract appear, and this occurred in only one eye. In all animals of group 04 the occurrence of cataracts was bilateral. The single cataract in group 01 is difficult to explain, but it might very well have been traumatic in origin. Gifford[6] described a type of cataract caused by heavy irradiation with radium and X rays. Tribenndeau and Belley[7] report cataracts, especially in young animals which have been treated with X rays, and von Hippel[8] reports a little more than 50 per cent of lenticular opacities in fetuses whose mothers had been irradiated. Frogé[9] produced opacities of the lens by irradiating young rabbits with X rays. In these studies, pathology of that nature did not occur in rabbits, but pathological conditions were found in the eyes of three of the dogs. Mucoid conjunctivitis was the most common change, and in one dog this progressed to a definite keratoconjunctivitis and then to a milky appearing corneal opacity.

In view of the evidence collected from neutron-irradiated rats, neutron radiation may be added to the various factors responsible for cataract formation.

4.2 Pathological Findings. The abnormalities which have been observed in rats, rabbits, and dogs subjected to repeated exposures of small doses of neutrons are of two major types. As was to be expected in an experiment of such long duration, so-called “spontaneous diseases” make up a major portion of the pathological changes observed. This is especially true in the case of rats where the experimental period occupied such a large part of the normal life span. Although these spontaneous diseases have been observed in both control and experimental animals, they become of importance when it can be shown that the treatment may account for a difference in incidence between the control group on one hand and the various experimental groups on the other. Of particular importance in this respect were the neoplastic diseases.

In addition to this type of change, there were observed other abnormalities in treated animals which were either absent in the controls or of such nature as to be classed as more direct effects of the treatment. Such changes were seen in the testes, ovaries, and hematopoietic organs.

(a) Testes. The male animals of all species which received 1.7 n/day showed marked testicular changes. With the exception of one rat that died early in the experiment, these changes were characterized by complete absence of spermatogenesis and by atrophy and often by distortion of the seminiferous tubules. This type of severe testicular atrophy was seen only in one case in group 03 and was not observed in the controls or in the two lower-dosage groups. The data available are not sufficient to allow a description of the histogenesis of this change, although the findings in rats and rabbits that died early in the year indicate that testicular injury occurred relatively early in animals exposed at the rate of 1.7 n/day. Marked, although incomplete, aspermatogenesis was seen in one rat after 46 treatments, and complete atrophy was observed in a rat after 46 treatments. In one rabbit after 72 treatments the testes showed a depletion of spermatogonia and primary spermatocytes, with less marked change in the more mature elements. Although no conclusive statement can be made on the basis of this one animal, these findings are compatible with the view that testicular changes resulting from repeated exposure to neutrons developed in the same way as those following acute X-ray exposure (Chap. 2).

Rats, rabbits, and dogs exposed to X radiation at the rate of 10 r/day and dogs exposed at the rate of 1 r/day (see Chap. 12) developed complete aspermatogenesis, as was observed in the 1.7-n group in this experiment. However, in most instances the findings in the X-rayed animals, like those here, represent an end result and do not necessarily indicate that the changes developed in the same way in both cases. Lampe and Hodges[10] exposed rabbit testes to neutrons and X rays with qualitatively similar results, and, although no histological details were reported, there was no indication that the genesis of the changes was different. In the studies with X rays and neutrons in this laboratory, there was only meager evidence that, with repeated exposure, the histogenesis of the testicular changes was the same. This evidence was afforded by one animal in each experiment which was available for study after a short period of exposure. The one rat exposed to a total of 39.60 n has been mentioned. In the experiment with X rays, one rabbit was available for study after 320 r was administered at the rate of 10 r/day. This rabbit showed depletion of the more primitive germinal cells, with the more mature elements intact.

In the case of exposure to X rays in repeated small doses, there was a definite difference noted in the different species with respect to testicular damage. Although rats and rabbits showed no effect at doses of 1.0 r/day and less, the dogs showed complete aspermatogenesis at 1.0 r and partial damage at 0.5 r. No such species difference was apparent from the results of exposure to neutrons, there being no clear-cut evidence that any of the three lower doses caused testicular damage in any species.

In rabbits and rats, in the control group and the three lower-exposure experimental groups, there was observed sporadically a type of testicular abnormality which was characterized by a depletion of spermatozoa and the more mature germinal cells in the seminiferous tubules and, in some cases, by the appearance of multinucleated giant cells. The latter were particularly conspicuous in rabbits. Changes of a similar type have been described: in senile rats by Wiesner,[11] in senile dogs by Smith,[12] in humans with infectious disease by Mills,[13] and in rats as a result of nutritional deficiencies and inanition.[14] The character of these changes was such as to suggest any one of a number of causes, and the incidence in this experiment was not such as to indicate that they were caused by exposure to neutrons.

The one case in which a rat exposed to 0.11 n/day showed complete aspermatogenesis is of unknown significance. Whether this change developed spontaneously or resulted from an unusual sensitivity to the effects of radiation cannot be determined.

(b) Ovaries. On the basis of breeding experiments, Lawrence and Tennant[15] report permanent sterility of female mice after exposure to neutrons in doses of the order of 130 to 300 r. Similar results were obtained with X rays. Although no breeding tests were carried out in the experiment reported in this chapter, there were definite changes observed in rats exposed to 1.7 n/day. The ovaries were small, and histologically they showed an absence of growing and Graafian follicles. Besides a few primordial follicles, there were many atretic follicles and often cellular accumulations resembling small corpora lutea. It is of interest that changes of a similar nature were observed in rats by Drips and Ford[16] following single doses of X rays.

The one female dog at the 1.7 n/day level showed changes of a similar type with extensive degeneration of follicles of an intermediate size.

The findings of ovarian changes with repeated exposure to neutrons and the absence of such changes are of some interest. This difference may well have been merely a quantitative one which was not apparent in the case of the testes, where both types of treatment resulted in complete atrophy in the highest exposure levels. This was one of a number of indications that the biological effects of 1.7 n/day were greater than those of 10 r/day.

(c) Hematopoietic Organs. The sensitivity of the hematopoietic organs to the effects of radioactivity of various types is well known, and in one of the first studies of the comparative effects of neutrons and X rays on mammals, Lawrence and Lawrence[17] used as a criterion of damage the peripheral-blood counts of rats. Following this study, Lawrence and Tennant[15] demonstrated hypoplastic changes in the hematopoietic organs of mice exposed to neutrons, which were qualitatively similar to those occurring after X irradiation.

Hypoplastic changes were observed in the hematopoietic system of all species at the 1.7 n/day level, but the distribution of these changes in the various organs of this system varied with the species.

Definite alterations in the spleen were observed in the rats and dogs that died during the period of exposure to 1.7 n/day. In the case of the rats, hypoplasia of the follicles occurred in all the animals in this group, and, in general, the degree of change was greater in those animals examined late in the exposure period. In dogs at this level the splenic changes were characterized by a similar decrease in the size and cellularity of the follicles. In addition, the dogs showed marked increase in the relative amount of connective-tissue elements of the spleen and wrinkling of the capsular margins of the sections, indicating an over-all atrophy of the organ.

With sections of lymph nodes available from only a very small percentage of the rats and rabbits used in this experiment, it was not possible to demonstrate any differences in the various groups with respect to changes in this organ in these particular species. In the dogs, however, representative sections from the regional lymph nodes were available, and marked changes were apparent in those animals exposed at the rate of 1.7 n/day. Without exception, the lymph nodes from dogs in this group showed severe hypoplasia of the lymphoid elements of the nodes and marked reduction in hematopoietic activity. In addition to the cellular depletion, the accumulation of blood and blood-filled macrophages in the lymph sinuses was observed in most cases. In this experiment, hemorrhagic lymph nodes accompanied the generalized hemorrhagic manifestations (Table 17.25). Similar changes were observed in dogs exposed repeatedly to X rays at the rate of 10 r/day (see Chap. 12), and Shouse, Warren, and Whipple[18] reported hemorrhage in lymph nodes of dogs after large single doses of X rays.

Bone-marrow changes (exclusive of those involved in neoplastic diseases), as observed by microscopic examination of the marrow in sections of vertebra and femoral epiphyses, were seen only in rabbits and dogs, and in these species at only the 1.7 n/day level. The abnormalities observed were characterized by a simple reduction in the hematopoietic elements of the marrow. In the more severely affected cases these changes approached complete aplasia of that part of the marrow examined. This marrow hypoplasia apparently involved all the marrow elements to the same degree as nearly as could be determined by this method of study. It was impossible to demonstrate any differences between the effect upon the erythroid elements and the myeloid elements.

Bone-marrow differential counts were made on marrow from the femoral shaft of all animals examined. In the case of animals that died during the exposure period, however, the marked variations within the various groups precluded any interpretation of the varied and slight differences between the groups. In the case of those animals in the three lower-exposure groups examined after exposure ceased, it was not possible to demonstrate any significant differences in the distribution of the marrow cellular types from one group to another or between the control and treated groups.

Table 17.25—Hemorrhage in Dogs in Group 04

Animal No.	Kidneys	Stomach	Small bowel	Large bowel	Lymph nodes	Heart	Other
03	+	++	+		+++	++	Epididymis ++
05		+*	+*				
04	+	+*	+*	+*	+++	++*	Lung +; skeletal muscle ++
02	++	+			+++	+*	Bone marrow +
01			+		+++	+*	Eye ++

*Gross observation only.

From the examination of the hematopoietic organs in animals exposed to X rays at the rate of 10 r/day (see Chap. 12), it was possible to demonstrate definite changes only in the bone marrow of dogs. The microscopic appearance of the marrow in these cases was essentially the same as that observed in this experiment. Hypoplastic changes in the marrow of rabbits, in the spleen of rats and dogs, and in the lymph nodes of dogs were less severe and less consistent at the 10-r level of the X-ray experiment in spite of the longer duration of exposure. These findings suggest a qualitative difference in the effects of that dosage and the dosage of neutrons used in this experiment. Hypoplastic changes have been demonstrated by many observers in the hematopoietic system following exposure to X rays, and further evidence that such changes may be produced by chronic X radiation was demonstrated by Henshaw[19] in mice exposed to repeated daily doses of X rays at 10, 15, and 25 r/day.

(d) Hemorrhage. Aside from the occurrence of cystlike hemorrhage areas in the adrenals of many of the rats that died during the experiment, a condition seen principally in female rats but which showed no apparent relation to the treatment, no evidence of an increased hemorrhagic tendency was observed except in the dogs exposed at the highest level. The organs most frequently involved were the kidneys, gastrointestinal tract, heart, and lymph nodes (see Table 17.25). In dogs exposed repeatedly to X radiation (see Chap. 12), hemorrhage was observed with significant frequency only at the 10 r/day level and was less consistently present and less extensive than that observed in this experiment. With X irradiation at the 10-r level, hemorrhage was limited, for the most part, to the gastrointestinal tract and lymph nodes. In both experiments these hemorrhagic manifestations occurred concomitantly with rather marked bone-marrow hypoplasia. The peripheral-platelet count was decreased in the dogs in both experiments (see Chap. 11).

Hemorrhage following exposure to large doses of X radiation administered acutely has been observed by many investigators, and Shouse, Warren, and Whipple[18] demonstrated a terminal reduction in circulating platelets in dogs that died with hemorrhagic manifestations after acute exposure to X rays. Recently, however, Allen and Jacobson[20] have demonstrated an additional factor in irradiated dogs, namely, the development of hyperheparinemia, and they consider this factor to be the primary one in postirradiation hemorrhagic disease. The results of the experiments described in this chapter shed no new light on the mechanism of the hemorrhage, but they do serve to demonstrate that hemorrhage may result from repeated exposure to relatively small doses of both X rays and fast neutrons. The data available are not sufficient to determine the relative importance of total dosage as opposed to rate of administration on the development of hemorrhagic tendencies.

(e) Neoplastic Diseases. Neoplasms were observed in rats and rabbits in this experiment. In the case of rats, such lesions occurred at all four levels of exposure as well as in the controls. In all groups, a variety of different types of tumor was observed. Table 17.26 summarizes these findings.

From the table, it seems apparent that the differences in the incidence of neoplasms, in general, in the control group and groups 01 and 02, are such as to be attributable to chance, considering the small number of cases necessary to account for these differences and the absence of an increase in incidence with increased dosage. The same is possibly true for any one of the four specific types of tumor. It will be seen from Tables 17.9 to 17.12 that, between the controls and groups

01 and 02, there was no appreciable difference in the death rate or the number of animals which died prior to the time of development of the earliest tumor. For this reason the control group and the two lower-dosage groups can be considered comparable as far as tumor incidence is concerned.

In group 03 the incidence of all types of tumors was greater than in the controls, and the total tumor incidence in this group was more than 3 times that in the controls. This figure represents the total number of animals with neoplasms, regardless of time and manner of death, and a comparison of this group with the controls should take into consideration certain differences between these two groups. In group 03 there were no deaths prior to the 158th treatment, whereas in the control group there were six potential tumor bearers that died prior to this time. However, the percentage of tumor bearers in rats surviving 158 treatments or longer was 13.4 per cent, which, for the purposes of this discussion, is not appreciably different from that obtained for the whole group. The total number of animals from the groups which died during the 1-year period was essentially the same.

Table 17.26 — Neoplasms in Rats

Group	00	01	02	03	04
Total cases	48	50	50	48	48
Leukemia, %	0	0	0	4.2	16.6
Reticulum-cell sarcoma, %	8.3	10.0	8.0	18.7	4.2
Other malignant tumors, %	0	4.0	0	12.5	0
Benign tumors, %	4.2	4.0	2.0	6.2	2.1
Total, %	12.5	18.0	10.0	41.6	22.9

In group 04 the total number of cases with neoplastic disease was considerably less than that in group 03 but more than in the controls (22.9 per cent as opposed to 12.5 per cent). This difference gains significance when the high death rate in group 04 from causes other than neoplasms is considered. This factor is undoubtedly at least partially responsible for the lower tumor incidence in group 04 than in group 03.

A comparison of the total tumor incidence in the above groups with that in the various groups of rats exposed to repeated doses of X rays reveals certain interesting similarities. Owing to the longer duration of the latter experiment, the number of neoplasms observed was greater in most groups than in the neutron experiment. In both experiments, however, the differences in total tumor incidence between the controls and the two lower experimental groups were not significant. The percentage incidence of neoplasms in the rats exposed to 0.11 n/day was essentially the same as in those exposed to 1 r/day. In the X-ray group the percentage of rats with neoplasms was about the same at the 10-r level as at the 1 r/day level and much greater than in rats exposed at the rate of 1.7 n/day. Again the survival time seems to have been an important factor, since the rats at the highest level in the X-ray experiment generally lived longer than those in group 04 in the neutron experiment.

Although the incidence of neoplasms, in general, has been found to be greater in certain of the irradiated groups than in the controls, the question remains as to whether or not this general increase may not have resulted from an increase in certain specific types of neoplasms. From observations made in these experiments and from the findings of other workers, certain types deserve special consideration.

Much evidence has been accumulated in recent years which supports the contention that the incidence of leukemia is affected by exposure to radiant energy. Furth and his associates[21–23] have made a number of interesting observations on irradiated mice. They have demonstrated an increase in "myelosis" in irradiated mice as well as an increase in the incidence of leukemia in mice treated with radiation and methylcholanthrene over that obtained when methylcholanthrene alone was used. They showed further that, when recipient mice were irradiated, the number of "takes" from a transmissible type of leukemia was increased. Henshaw[19,24] has likewise found an increase in leukemia in mice after repeated exposure to X rays. In addition to this experimental evidence, it has been shown by March,[25] who analyzed the records on the causes of death in physicians over a period of 15 years, that there was a significantly greater number of deaths from leukemia in radiologists than in nonradiological physicians.

In the light of these findings in other species and considering the fact that, from the many hundreds of rats examined in this laboratory, not a single case of leukemia has been observed except in those exposed to X rays or neutrons, these results serve as strong evidence that exposure to radiation was responsible for the increase in observed cases of leukemia in rats. It should be kept in mind, however, as has been stated elsewhere in this volume, that there is nothing to suggest that the irradiation caused the leukemia since the findings may merely demonstrate an increase in the rate of development of this condition.

Whereas the high incidence of neoplasms in group 04 was largely due to the large number of cases with leukemia, the incidence of tumors excluding leukemia in group 03 was still approximately three times that in the controls. Although the most common tumor was the reticulum-cell sarcoma (this was also the most common tumor in controls), it is not possible, considering the small number of animals showing a specific type of tumor, to attribute the over-all increase to any single tumor type or to any combination of tumor types. Benign mammary tumors which showed an increase in incidence with increasing dosage in the X-ray experiment were relatively infrequent in occurrence in this experiment and showed no apparent increase in any irradiated group. This marked difference in the incidence of mammary tumors in these experiments is probably due to the difference in duration since most tumors of this type occurred during the second year of the X-ray experiment. Another type of tumor which was of some interest was the osteogenic sarcoma in one case in group 03. This type of tumor has been produced in several species by a number of workers using radiant energy. The subject of experimental bone tumors from radiant energy has been reviewed by Gates.[26] Of course, little significance can be attached to the single case observed here, especially since one case of osteogenic sarcoma was observed in a control rat in the X-ray experiment.

Tumors were observed in two rabbits exposed at the rate of 1.7 n/day. These tumors were of the same type, and the consensus among a number of pathologists is that this tumor has the appearance of a nonpigmented melanoma. Regardless of the exact type of tumor, the fact remains that malignant tumors which were essentially the same in histological appearance developed in two of the three rabbits receiving the greatest total exposure. Even though the evidence is insufficient to support any definite conclusions concerning the relation of the treatment to the development of these tumors, the above-mentioned facts point to the need for further investigation of this problem.

(f) Infectious Diseases. Diseases of an infectious nature occurred with significant frequency only in rats. The important types of diseases in the rat were pulmonary infection and, to a lesser extent, paratyphoid infection in the liver, spleen, and large bowel. The incidence of such conditions was essentially the same in the three lower-dosage groups as in the controls. In group 04, however, the incidence of infectious diseases was more than twice that in all the controls. Since, in approximately 40 per cent of the group 04 rats, the only lesions to which death could be attributed were of an infectious nature, it must be assumed that this increase in infection was, in part, responsible for the high mortality in this group.

5. SUMMARY

Rats, rabbits, and dogs have been subjected to neutron radiation at dosage levels of 0.012, 0.06, 0.11, and 1.7 n/day, 6 days a week, for a continuous period of 1 year. Clinical, pathological, and hematological changes have been observed.

5.1 Clinical Findings. Dosage levels of 0.012, 0.06, and 0.11 n/day produced no observable clinical effects or changes which could be demonstrated grossly during post-mortem examination in any of the animals. In contrast, all animals receiving the highest dosage level, i.e., 1.7 n/day, showed pronounced changes. Marked loss in weight, reduced food intake, and a general debilitated condition resulted. Bilateral cataracts occurred in the rats. Mucoid conjunctivitis, keratoconjunctivitis, and corneal opacities were found in the dogs. The spleen and testes of all animals in this group were characteristically found to be reduced in size at post-mortem examination.

5.2 Pathological Findings. The pathological findings fall into five general categories.

(a) Spontaneous Infectious Diseases. In these diseases, pulmonary infection and a paratyphoid infection were the most frequently observed. Such diseases were not significantly increased in animals exposed at the rates of 0.012, 0.06, and 0.11 n/day. The large number of rats in the 1.7 n/day group that died with severe infectious involvement supports the contention that infection which was presumably related to exposure was, in part, responsible for the high death rate in this group.

(b) Neoplasms. Neoplastic diseases which included a variety of different types were more than 3 times as common in rats exposed at the rate of 0.11 n/day as in the controls. Two rats, or 4.2 per cent of all rats in the former group, showed a leukemoid picture, whereas no such cases were observed among the controls. In rats exposed at the rate of 1.7 n/day, neoplasms were more common than in the controls (22.9 per cent as opposed to 12.5 per cent) in spite of the high mortality in the former group from causes other than neoplasms. Leukemoid changes which occurred in 16.6 per cent of this group accounted, to a large extent, for this relatively high incidence of neoplasms.

Neoplasms among the rabbits were limited to two cases that died late in the exposure period; these two showed malignant tumors diagnosed as nonpigmented melanomata.

(c) Hypoplastic Changes in Hematopoietic Organs. Hypoplasia of the spleen was observed in rats and dogs exposed at the rate of 1.7

n/day. The bone marrow was hypoplastic in dogs and rabbits at this same level. Sections of lymph nodes were available from so few rats and rabbits that the findings could not be interpreted. However, in dogs at the 1.7-n level there was generalized marked hypoplasia of regional nodes.

Bone-marrow differential counts on dying animals showed such wide variations within groups that it was not possible to interpret differences between groups. In cases where animals were available for sacrifice at the termination of the experiment (all species in the three lower-dosage groups and in controls), there was no apparent difference between groups in the percentage distribution of marrow cellular elements.

(d) Atrophic Changes in Gonads. Marked atrophic changes were consistently observed in the testes of all species exposed at the rate of 1.7 n/day. Atrophy and a reduction in the number of ovarian follicles occurred in rats at this level and in the only female dog from which sections of ovary were available. No female rabbits were used in this experiment. No significant changes were observed in the gonads of any species at the three lower dosage levels.

(e) Hemorrhage. Hemorrhagic manifestations were observed in all dogs exposed at the rate of 1.7 n/day. The organs most commonly involved were the lymph nodes, heart, stomach, small bowel, and kidneys.

5.3 Hematological Findings. The extreme variability in the counts of many of the cellular elements of the peripheral blood tended to obscure all except very well-defined trends. The observed effects may be summarized briefly as follows:

1. The most marked response to chronic neutron irradiation was a reduction in the number of lymphocytes in the peripheral blood. It was observed in dogs of all treatment groups and in rabbits and rats of the 1.7 n/day group.

2. Neutrophils were depressed within 2 weeks after exposure to 1.7 n/day in dogs and after 8 weeks of the same exposure in rabbits. No other groups showed significant neutrophil trends.

3. Erythrocyte depressions were the last to occur and were noted in all animals after 32 to 35 weeks of irradiation at the rate of 1.7 n/day.

4. Platelets and reticulocytes were not significantly altered in any of the animals exposed to neutron irradiation.

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